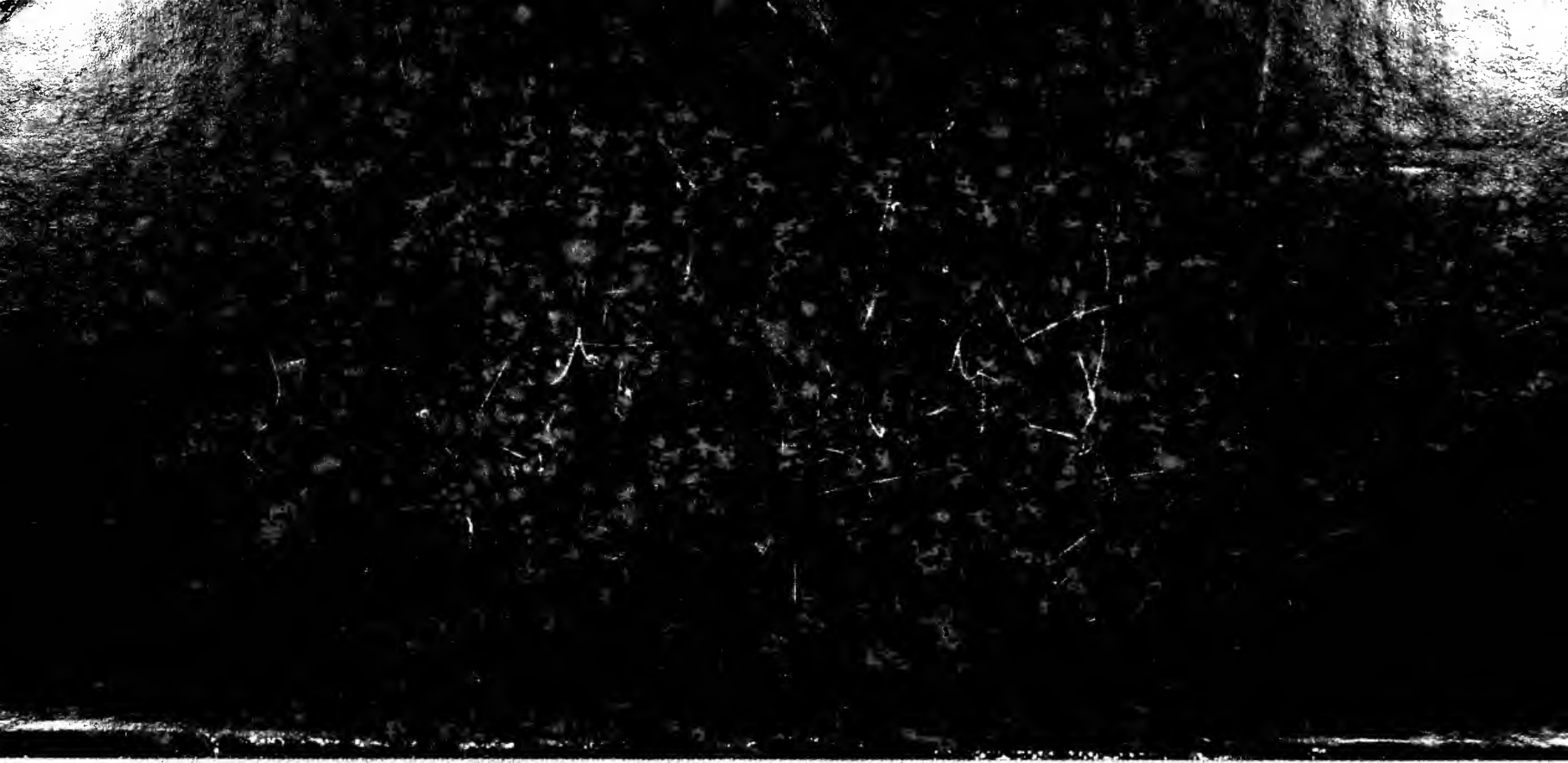
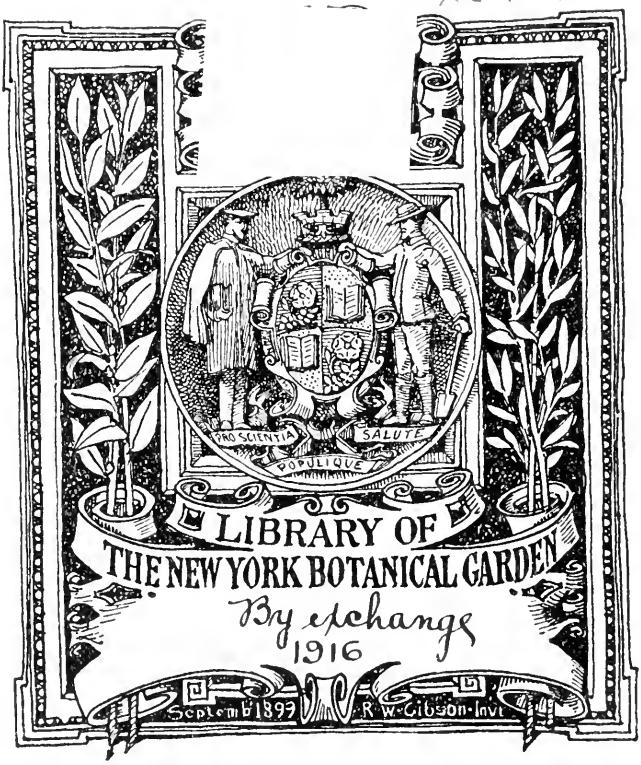



PRO SCIENTIA
SALUTE
POPULIQUE
LIBRARY OF
THE NEW YORK BOTANICAL GARDEN
By exchange
1916
September 1899
R. W. Gibson. Invt.

JOURNAL OF AGRICULTURAL RESEARCH

VOLUME VI

APRIL 3, 1916—SEPTEMBER 25, 1916

DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

CONTENTS

ERRATA

Page 151, line 25, "Sphaeropsideae" should read "Sphaerioidaceae."

Page 156, "*Penicillium camemberti*, var. *rogri*" should read "*Penicillium camemberti*, var. *rogeri*."

Page 296, Pl. XXXIII, figs. 3 to 15. The magnification of the illustrations should be half that stated in the legend.

Page 303, line 17, "Plate XXXVI, figures 1 to 4" should read "Plate XXXVII, figures 1 to 4" and "In figure 4, Plate XXXVI" should read "In figure 4, Plate XXXVII."

Page 318, Table IV, under head "General remarks," "rooting" should read "shooting."

Page 337, Table II, 4th column, "Phenolized defibrinated blood 3895 (unwashed)" should read "Phenolized defibrinated blood 3895."

Page 377, last line, 2d paragraph, "winged" should read "wiugless."

Page 384, Table I, 6th column, "Current (milliampere minutes)" should read "Current (milliamperes)."

Page 388, line 13 from bottom, omit "with humidity at 57."

Page 419, line 25, "The twelve-spotted (or squash) lady beetle" should read "The squash lady beetle."

Page 419, line 28, "(*Crepidodera cucumeris*)" should read "(*Epitrix cucumeris*)."

Page 459, lines 2 and 24, omit "Three."

Page 471, line 4, "*Aleurodes mori* Ckll." should read "*Aleurodes mori*, var. *arizonensis* Ckll."

Page 762, Table I, first column, "*Medicago arbica*" should read "*Medicago arabica*."

Page 791, Table XIV, 1st column, "(p. 23)" should read "(p. 783)."

Page 865, legend under figure 5, "The solid black line, etc.," should read "The hatched line."

Page 866, legend under figure 6, omit sentences 2 and 3.

Page 881, line 6 from bottom, "April" should read "May."

ILLUSTRATIONS

PLATES

Relation of Carbon Bisulphid to Soil Organisms and Plant Growth

Climatic Conditions as Related to Cercospora beticola

Oviposition of Megastigmus spermotrophus in the Seed of Douglas Fir

CITRUS CANKER

INSECT INJURY TO COTTON SEEDLINGS

WATERMELON STEM-END ROT

CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS

THE DISEASE OF POTATOES KNOWN AS "LEAK"

SOME PROPERTIES OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO

LIFE CYCLES OF THE BACTERIA

α-CROTONIC ACID, A SOIL CONSTITUENT

TEXT FIGURES

CLIMATIC CONDITIONS AS RELATED TO CERCOSPORA BETICOLA

CITRUS CANKER

DETERMINATION OF STEARIC ACID IN BUTTER FAT

LIFE HISTORY AND HABITS OF TWO NEW NEMATODES PARASITIC ON INSECTS

EFFECT OF PASTEURIZATION ON MOLD SPORES

EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM

HYPODERMA DEFORMANS, AN UNDESCRIBED NEEDLE FUNGUS OF THE WESTERN YELLOW PINE

COMPARATIVE STUDY OF THE ROOT SYSTEMS AND LEAF AREAS OF CORN AND THE SORGHUMS

WOOLLY PEAR APHIS

STIMULATING INFLUENCE OF ARSENIC UPON THE NITROGEN-FIXING ORGANISMS OF THE SOIL

STORAGE-ROTS OF ECONOMIC AROIDS

LARVAL CHARACTERS AND DISTRIBUTION OF TWO SPECIES OF DIATRAEA

THE DISEASE OF POTATOES KNOWN AS "LEAK"

LIFE CYCLES OF THE BACTERIA

MOTTLE-LEAF OF CITRUS TREES IN RELATION TO SOIL CONDITIONS

AGRICULTURAL VALUE OF IMPERMEABLE SEEDS

LIFE-HISTORY STUDIES OF CIRPHIS UNIPUNCTA, THE TRUE ARMY WORM

THERSILOCHUS CONOTRACHELI, A PARASITE OF THE PLUM CURCULIO

EFFECT ON PLANT GROWTH OF SODIUM SALTS IN THE SOIL

APHIDOLETES MERIDIONALIS, AN IMPORTANT DIPTEROUS ENEMY OF APHIDS

INFLUENCE OF BARNYARD MANURE AND WATER UPON THE BACTERIAL ACTIVITIES OF THE SOIL

PROGRESSIVE OXIDATION OF COLD-STORAGE BUTTER

BACTERIOLOGICAL STUDIES OF A SOIL SUBJECTED TO DIFFERENT SYSTEMS OF CROPPING FOR 25 YEARS

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.—XV. DWARF EGGS

Vol. VI APRIL 3, 1916 No. 1

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., APRIL 3, 1916 NO. 1

RELATION OF CARBON BISULPHID TO SOIL ORGANISMS AND PLANT GROWTH[1]

By E. B. FRED,
Agricultural Bacteriologist, Agricultural Experiment Station of the University of Wisconsin

INTRODUCTION

In a previous publication concerning the action of carbon bisulphid (CS_2) on bacteria and plants data were presented to show the beneficial effect of this substance on the soil flora (1).[2] The increased plant growth following the addition of carbon bisulphid in many cases is enormous. For example, a small application often causes an increase in yield from 100 to 200 per cent. It is impossible to account for this remarkable gain on the assumption that the only action of the carbon bisulphid is that of added plant food. It was found, as has been noted by many investigators (5, 6, 11, 12), that this volatile antiseptic exerts a very decided effect on the micro-organisms of the soil. As measured by plate counts, there is at first usually a great decrease in numbers, followed by a period of excessive increase, the total numbers far exceeding those that ordinarily exist. In certain cases carbon bisulphid has not only failed to cause an increase in plant growth, but has, on the contrary, caused a decrease.

Search has been made by many investigators for a satisfactory explanation of this peculiar action of carbon bisulphid. Many theories have been advanced. Concerning these theories so much has been written that a detailed discussion of the literature seems unnecessary. Indeed, it would be impossible within the limited scope of this paper to present a summary of the various explanations. One point is very prominent in nearly all of the publications: The action of carbon bisulphid is varied. Because of the interest attached to this problem, it was arranged to study some of the factors that might influence the action of carbon bisulphid. The experiments described in this paper are discussed under three main heads: First, the effect of varying amounts of carbon bisulphid; second, the effect of carbon bisulphid on various plants; and third, the effect of carbon bisulphid in various soils. In all of this work fresh field soil and commercial carbon bisulphid were used. Some of the experiments represent a combined study of the effect on both the lower and higher forms of plant life.

[1] Published with permission of the Director of the Wisconsin Agricultural Experiment Station.
[2] Reference is made by number to "Literature cited," p. 18–19.

EXPERIMENTAL METHODS

Commercial carbon bisulphid was poured into small holes in the soil, and these were covered immediately. The soil was sieved and potted in 2-gallon jars and the moisture maintained at half saturation. Changes in the soil flora were determined at regular intervals by plate counts of the number of bacteria and dilution counts of the number of active protozoa. The formation of ammonia and nitrates was measured at regular intervals.

The following plants were used: Buckwheat (*Fagopyrum fagopyrum*), clover (*Trifolium pratense*), corn (*Zea mays*), mustard (*Sinapis alba*), oats (*Avena sativa*), and rape (*Brassica napus*). In many of the experiments a first and a second crop were grown.

EFFECT OF CARBON BISULPHID ON THE NUMBER AND ACTIVITY OF SOIL ORGANISMS

Eight jars were filled with Miami silt-loam soil from the Experiment Station farm. These were arranged in duplicate and treated as follows: (1) Control, untreated; (2) 2 per cent of carbon bisulphid; (3) 2 per cent of carbon bisulphid, evaporated; (4) 2 per cent of carbon bisulphid, evaporated, and reinoculated with 5 per cent of the original soil.

Twenty-four hours after treatment the soil in the evaporated series was spread out on sterile paper and the volatile antiseptic allowed to escape. At the end of the second 24-hour period the soil was put back into the jars. In order to prevent any contamination, the jars were covered with a double layer of cheesecloth and nonabsorbent cotton. This cover should allow free access of air without much danger of contamination. At regular intervals the covers were removed and samples drawn for analysis. The results of these determinations are presented in Tables I and II.

NUMBER OF ORGANISMS

BACTERIA.—In Table I are shown the number of bacteria in 1 gm. of soil at different times and under the different conditions.

TABLE I.—*Effect of carbon bisulphid on number of bacteria*

Time.	Bacteria per gram of dry soil.			
	Control.	2 per cent of carbon bisulphid.	2 per cent of carbon bisulphid evaporated.	2 per cent of carbon bisulphid evaporated + 5 per cent of soil from control.
Days.				
1	11, 496, 000	1, 965, 000	2, 260, 000	2, 358, 000
3	22, 010, 000	23, 975, 000	8, 254, 000	12, 480, 000
5	20, 635, 000	25, 253, 000	27, 416, 000	95, 499, 000
9	14, 739, 000	36, 651, 000	61, 904, 000	.....
13	16, 115, 000	90, 473, 000	98, 850, 000	80, 420, 000
21	19, 508, 000	60, 149, 000	71, 257, 000	52, 495, 000
25	18, 272, 000	68, 276, 000	86, 483, 000	64, 570, 000
29	15, 346, 000	90, 645, 000	84, 272, 000	38, 495, 000
60	12, 372, 000	58, 101, 000	60, 000, 000	30, 000, 000

At first the antiseptic causes a great reduction in the number of organisms capable of developing on Heyden agar. The period of depression lasts for only a short time—in this experiment about five days. From that time until the end of the test the number of organisms in the treated series far exceeded that of the control. The highest number in the carbon bisulphid evaporated and unevaporated soil occurred about the thirteenth day; while the carbon bisulphid evaporated soil plus control soil gave the highest count on the fifth day. At the time of the last count, 60 days after carbon bisulphid was added, the organisms in the treated series far exceeded those in the original soil. Apparently the effect of carbon bisulphid on the number of bacteria is noticeable for a long period of time.

If the results of the counts with carbon bisulphid unevaporated are compared with those of carbon bisulphid evaporated, it appears that no very marked difference exists. The greatest reduction in numbers occurred in soils with the carbon bisulphid evaporated. It is significant that soil with carbon bisulphid evaporated should prove more injurious to micro-organisms than the unevaporated. This agrees with Gainey (2, p. 592), who reports that the combined effect of the two processes seemed more injurious to nitrification than treatment with carbon bisulphid unevaporated.

After the thirteenth day the treated and reinoculated soil did not show as many organisms as the treated series. This difference is shown very distinctly in Plate I, which is reproduced from a photograph of a number of colonies developing on agar. Four parallel plates were made from the same dilution of each soil.

On this date samples were also drawn for ammonification tests. The purpose of this was to measure the rate of the decomposition of casein in the various series, and 1 per cent of casein was added to the soil and the ammonia determined after 12 and 24 hours. The beneficial effect of carbon bisulphid on ammonification is very evident. If after 12 hours the untreated is 100, then carbon bisulphid unevaporated is 154, carbon bisulphid evaporated is 212, and carbon bisulphid reinoculated is 190.

After 24 hours the untreated is equal to 100, carbon bisulphid unevaporated is 149, carbon bisulphid evaporated is 171, and carbon bisulphid reinoculated is 153. The data show very clearly that casein is decomposed more rapidly in treated than in untreated soils. This difference is most prominent in the 12-hour tests.

PROTOZOA.—Counts at the beginning showed the presence of protozoa in dilutions representing 1 to 1,000 gm. of soil (13, p. 626). Two weeks after treatment the soils were recounted. At this time numerous small flagellates were found in dilutions of 1 to 1,000. It is evident that the different treatments with carbon bisulphid had not seriously injured this group of organisms.

AZOTOBACTER.—One month after treatment with carbon bisulphid, qualitative tests were made. The Azotobacter organisms were found in all soils. The brown film of Azotobacter from the treated soils was not so profuse as that from the original soil.

ALGÆ.—In order to estimate the number of algæ, dilution tests were made. These cultures were incubated for 30 days. The smaller forms were found in great numbers in all of the soils.

The important facts in these data are (1) that the volatile antiseptic fails to remove these larger soil organisms and (2) that the smaller forms of bacteria are only temporarily reduced. The decrease in numbers is soon followed by a period of excessive growth.

ACTIVITY OF ORGANISMS

A rapid multiplication of bacteria should naturally be followed by a parallel increase in decomposition products. Accordingly samples for analysis were drawn from the jars used in the previous experiment. The results of these periodic analyses are presented in Table II.

TABLE II.—*Effect of carbon bisulphid on ammonia and nitrate content of soil*

Time.	Nitrogen per 100 gm. of dry soil.							
	Ammonia.				Nitrate.			
	Control.	2 per cent of carbon bisulphid.	2 per cent of carbon bisulphid evaporated.	2 per cent of carbon bisulphid evaporated + 5 per cent of soil from control.	Control.	2 per cent of carbon bisulphid.	2 per cent of carbon bisulphid evaporated.	2 per cent of carbon bisulphid evaporated + 5 per cent of soil from control.
Days.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
At beginning	1. 60	1. 60	1. 60	1. 60	2. 66	2. 66	2. 66	2. 66
30	1. 68	5. 27	5. 41	4. 71	3. 35	2. 50	2. 00	5. 55
45	2. 38	8. 40	7. 70	4. 90	3. 75	2. 70	2. 50	5. 66
60	2. 59	5. 43	5. 32	2. 31	4. 00	2. 81	2. 50	4. 50
75	1. 85	5. 60	.....	2. 10	3. 20	2. 40	2. 60	5. 00
90	2. 94	4. 06	4. 06	2. 24	4. 00	5. 00	3. 32	6. 66

In the soils treated with carbon bisulphid there is a very decided accumulation of ammonia nitrogen. If the figures of Table I are compared with those of Table II, ammonia production, it will be seen that an increase in the number of bacteria within a certain range results in a gain in ammonia. After 30 days the amount of ammonia nitrogen in the treated soils averaged more than three times that in the original soil. After 60 days the ammonia content in the carbon bisulphid and carbon bisulphid evaporated soil was about double that of the control, while in the carbon bisulphid evaporated plus 5 per cent fresh soil it was

less. From the data it appears that reinoculation prevents large accumulations of ammonia. This is no doubt due to the oxidation of ammonia by the nitrifying bacteria. The figures of the last column (nitrate accumulation) support this statement. A stimulation of ammonification is still noticeable at the end of 3 months.

The nitrate-forming bacteria apparently do not recover so rapidly from carbon bisulphid treatment as the ammonia-producing organisms; consequently, there is no increase in nitrates until the end of 3 months. An exception to this is noted in the reinoculated soil. Here the activity of the nitrifying bacteria is evident 30 days after inoculation.

In order to ascertain, as nearly as possible, the effect of carbon bisulphid on the soluble nitrogen of the soil, the figures of Table II, ammonia and nitrate nitrogen, were combined in Table III.

TABLE III.—*Effect of carbon bisulphid on soluble nitrogen*

Time.	Ammonia and nitrate nitrogen per 100 gm. of dry soil.			
	Control.	2 per cent carbon bisulphid.	2 per cent carbon bisulphid evaporated.	2 per cent carbon bisulphid evaporated +5 per cent of soil from control.
Days.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
At beginning	4. 26	4. 26	4. 26	4. 26
30	5. 03	8. 47	7. 41	10. 26
45	5. 13	11. 10	10. 20	10. 56
60	6. 59	8. 24	7. 82	6. 87
75	5. 05	8. 00	.....	7. 10
90	6. 94	9. 06	7. 38	8. 90

From the data in this table it is very evident that carbon bisulphid causes a large increase in ammonia and nitrate nitrogen. There seems to be very little difference between the effect of the various treatments of carbon bisulphid on the formation of ammonia and nitrate nitrogen. When compared with the control soil, it will be seen that 45 days after treatment the carbon-bisulphid soils contain more than twice as much soluble nitrogen. The higher ammonia and nitrate content is very marked 90 days after treatment. A repetition of this experiment gave similar results.

A review of the data in Tables II and III shows very clearly that carbon bisulphid in Miami soil increases the total soluble nitrogen—namely, ammonia and nitrates. One interesting fact that appears from a comparison of the ammonia and nitrate content is that these two substances are to a certain degree inversely proportional.

EFFECT OF CARBON BISULPHID ON THE HIGHER AND LOWER FORMS OF PLANT LIFE

From the results of the preceding experiments it seems that carbon bisulphid should exert a beneficial effect on the growth of higher plants. At first this should be most marked with ammonia-feeding plants, and later with nitrate-feeding plants. Unfortunately it is not possible to secure plants that feed entirely on nitrates or ammonia. For this reason it was thought best to study the relation of carbon bisulphid to the growth of several different plants. Accordingly a combination study of the effect of carbon bisulphid on higher plants and on bacteria was made. A wide range of soil types, as well as different higher plants, was used.

Before entering upon a study of the relation of carbon bisulphid to soil type and various plants, it was desired to obtain some idea of the influence of various amounts of carbon bisulphid on plant growth. The procedure was as follows: Ten kgm. of field soil (Miami silt loam) were placed in each of sixteen 2-gallon jars. The carbon bisulphid was added in varying amounts, from 0.5 per cent to 2 per cent. It was poured into holes in the soil. These holes were closed immediately and the water increased to half saturation. In order to overcome the injurious effect of carbon bisulphid, the jars were then allowed to stand for two weeks before planting.

CORN AND MUSTARD IN MIAMI SILT LOAM

The results of the test with corn and mustard are given in Table IV. It is evident from the data of the table that these plants do not respond alike to carbon bisulphid.

TABLE IV.—*Effect of varying amounts of carbon bisulphid on the growth of corn and mustard*

No.	Soil.	Carbon bisulphid added.	Weight of corn.			Weight of mustard.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami.	Control.	75	20	22.5	58	9.5	9.25
2	...do...	Control.	80	25		49	9	
3	...do...	0.5	82	18	19.5	69	13	12.50
4	...do...	.5	83	21		72	12	
5	...do...	1	132	28	19.5	95	13	12.50
6	...do...	1	22	11		75	12	
7	...do...	2	85	21	24	105	16	16.50
8	...do...	2	125	27		112	17	

In all concentrations except 2 per cent, carbon bisulphid injured the growth of corn. Mustard, on the other hand, was greatly benefited by the carbon-bisulphid treatment. An increased growth was observed from all concentrations. The maximum gain was noted with 2 per cent of carbon bisulphid. This beneficial effect on mustard is very evident from Plate II, figure 1. If this increase in growth is due to the larger

amount of soluble nitrogen as ammonia or nitrate, then corn and mustard should behave much alike. The nitrogen-feeding power of these plants has been studied by Krüger (8), Gerlach and Vogel (3), and others. It is supposed that both corn and mustard are heavy nitrogen-feeding crops, able to take nitrogen either in the form of ammonia or nitrate.

BUCKWHEAT, CORN, AND OATS IN MIAMI SILT LOAM

In order to decrease the factor of individual variation, four parallel jars of Miami silt loam were used in each series in the following experiment. For the second crop these were subdivided into sets of two each. After the first crop was harvested, the soil and roots were thoroughly mixed and the jars replanted. The rotation was as follows: First crop, buckwheat; second crops, corn and mustard; first crop, corn; second crop, buckwheat; first crop, oats; second crops, corn and mustard. In Tables V, VI, and VII are presented the results of these experiments.

TABLE V.—*Effect of carbon bisulphid on the growth of buckwheat and corn*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, buckwheat.			Weight of second crop, corn.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami	Control.	90	15.5	19	152	28.5	31
2	...do...	Control.	97	18		160	33.5	
3	...do...	Control.	121	22.2		.....	.....	
4	...do...	Control.	126	20.5		.....	.....	
5	...do...	2	124	23	24.5	169	34	32.7
6	...do...	2	145	26.5		136	31.5	
7	...do...	2	127	23		.....	.....	
8	...do...	2	126	25.5		.....	.....	

The yields of buckwheat and corn are given in Table V. The weights of the mustard were lost. Buckwheat gave an increase in the treated soil, while corn (the second crop) did not show any improvement. Determinations of ammonia present at the time the buckwheat was cut (three months after treatment) resulted as follows: Ammonia—if control is 100, then carbon bisulphid treated is 192. Nitrate—if control is 100, then carbon bisulphid treated is 28. The antiseptic increases ammonia, but decreases the nitrate content of soil. The results of investigation show that buckwheat feeds largely on nitrate nitrogen (9), while corn is supposed to be able to take its nitrogen in the form of ammonia. A difference in nitrogen-feeding power can not be used to explain the unequal behavior of these plants toward carbon bisulphid. Although the weights of the mustard crop were not kept, the action of the carbon bisulphid was evident. There was a decided gain in the growth of plants in the treated series.

From the data of Table VI it is obvious that carbon bisulphid has very little effect on corn (first crop) or buckwheat (second crop).

TABLE VI.—*Effect of carbon bisulphid on the growth of corn and buckwheat*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, corn.			Weight of second crop, buckwheat.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami.	Control.	480	85		115	26	
2	...do...	Control.	440	82	84.7	125	27	23.5
3	...do...	Control.	500	90		100	22	
4	...do...	Control.	410	82		95	19	
5	...do...	2	380	77		128	27	
6	...do...	2	460	85	83	87	17	20.5
7	...do...	2	410	83		95	20	
8	...do...	2	460	86		87	18	

Table VII gives the effect of this volatile antiseptic on oats (first crop) and corn (second crop). The former showed an increase in growth in the treated soil; the latter was not affected.

TABLE VII.—*Effect of carbon bisulphid on the growth of oats and corn*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, oats.			Weight of second crop, corn.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami.	Control.	172	46.5		166	40	
2	...do...	Control.	184	51	48.3	132	31	36
3	...do...	Control.	171	46.7		118	29	
4	...do...	Control.	182	49		180	45	
5	...do...	2	200	59		155	37	
6	...do...	2	205	59	57.8	161	38	37
7	...do...	2	197	57.7		152	37	
8	...do...	2	192	57.5		135	36	

A general consideration of the data shows that corn in this soil type is apparently indifferent toward carbon bisulphid. Buckwheat, oats, and mustard were all benefited by the antiseptic.

BUCKWHEAT, MUSTARD, OATS, AND CORN IN DIFFERENT SOILS

The experiment with buckwheat, mustard, corn, and oats was a combination study of the effect of carbon bisulphid on bacterial activity and plant growth in three different soils. The first series contained Miami silt loam, the second series Miami soil diluted one-half by volume with sand, and the third series sand alone. According to chemical analysis, Miami silt loam is fairly rich in organic matter, nitrogen, potassium, and phosphorus. Of the three fertilizing elements, phosphorus perhaps is present in the smallest amount. The quantity of soil and its treatment was similar to that of the preceding experiment except that the treated jars were kept tightly covered with parchment paper. One month after the carbon bisulphid was added, these were removed. By

this means it was hoped to prevent a rapid volatilization of the antiseptic. The jars were not planted until three months after treatment.

At the beginning and at intervals of one, two, and three months bacterial activity was measured. Naturally, under the conditions of this experiment, carbon bisulphid proved very drastic. A great reduction in the number of bacteria, without any increase until the second month, was noted. The relation of carbon bisulphid to the number of bacteria was about the same in all three series. In the more compact type, Miami silt-loam soil, the carbon bisulphid proved most injurious to numbers, and consequently the period of increase was much later. Of the three soils, the treated sand showed the greatest proportional gain in number of bacteria.

Because of the severe nature of the carbon-bisulphid treatment, it was thought that probably the protozoa would be destroyed or the number greatly diminished. This was not the case, however, as protozoa were found in great numbers in both the treated and untreated soil.

Three months after treatment the jars were divided into two series and planted. The weights of the first and second crops are given in Tables VIII and IX.

TABLE VIII.—*Effect of carbon bisulphid on the growth of buckwheat and mustard in different types of soil*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, buckwheat.			Weight of second crop, mustard.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami silt loam.....	Control.	123	22. 5	21. 5	12. 0	3. 4	3. 3
2	.....do.....	Control.	107	20. 5		10. 0	3. 3	
3	.....do.....	2	119	25. 0	24. 0	24. 5	5. 2	7. 3
4	.....do.....	2	114	23. 0		41. 5	9. 5	
5	Half Miami silt loam, half sand.	Control.	74	15. 5	15. 25	21. 0	3. 75	3. 67
6	.....do.....	Control.	72	15. 0		17. 0	3. 60	
7	.....do.....	2	76	18. 0	17. 0	21. 5	4. 5	4. 25
8	.....do.....	2	78	16. 0		17. 0	4. 0	
9	Sand.....	Control.	20	2. 5	2. 75	4. 0	0. 4	.45
10	.....do.....	Control.	21	3. 0		4. 5	0. 5	
11	.....do.....	2	21. 5	3. 0	3. 00	17. 5	1. 2	.90
12	.....do.....	2	21. 5	3. 0		5. 5	. 6	

The figures of the buckwheat crop show the same general increase as noted in a previous experiment. Although not great, the gain in the treated series is consistent in all three soils.

The residual crop of mustard responded to a very marked degree to the carbon bisulphid treatment. In Miami silt loam the yield from the treated soil exceeded that of the control by more than 100 per cent. The gain in weight of oats in the treated soils was not so great, while the second-crop corn showed a loss (Table IX).

TABLE IX.—*Effect of carbon bisulphid on the growth of oats and corn in different types of soil*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, oats.			Weight of second crop, corn.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami silt loam.....	Control.	162	47	47.7	114	25	24
2	.....do.....	Control.	180	48.5		103	23	
3	.....do.....	2	157	45.5	49	77	18.5	19.2
4	.....do.....	2	190	52.5		87	20	
5	Half Miami silt loam, half sand.....	Control.	82	26.5	27.2	76	16	15
6	.....do.....	Control.	85	28		56	14	
7	.....do.....	2	85	27.5	28	52	13	14
8	.....do.....	2	82	28.5		64	15	
9	Sand.....	Control.	.....	.....	.....	.....	.....	.....
10	.....do.....	Control.	18	6	6	12	4	4
11	.....do.....	2	18	5.8	5.5	14	5	4.5
12	.....do.....	2	15	5.2		13.5	4	

The results of the nitrate determinations agree with those obtained in previous experiments. At the time of planting the carbon-bisulphid soils were lower in nitrate but higher in ammonia than the original soil.

The data from Tables VIII and IX show that carbon bisulphid has a much more beneficial effect on mustard than on any other crop. Buckwheat and oats are benefited, but not so markedly as mustard. Corn fails to show any improvement from treatment with carbon bisulphid.

EFFECT OF CARBON BISULPHID ON BUCKWHEAT AND RAPE IN VARIOUS SOILS

The five soil types selected for the study of the effect of carbon bisulphid on buckwheat and rape in various soils ranged all the way from a very compact red clay to an open, sandy soil. After treating with 2 per cent of carbon bisulphid the soils were allowed to stand for three months before planting. Bacteria counts and nitrate determinations were made at the beginning and after two and three months. The effect of the carbon bisulphid on the total number of bacteria is very evident. In every case the carbon-bisulphid soil contained the most bacteria. The maximum gain occurred in the clay-loam soil, the minimum in the Norfolk sand. The increase due to the treatment was greatest after two months.

Here, again, the treated soils gave a much lower nitrate content than the controls. It seems safe to say that a rapid increase in numbers of bacteria in a carbon-bisulphid soil is followed by a decrease in the amount of nitrates.

Three months after treatment the soils were planted to buckwheat. Growth was slow at first, especially in the carbon-bisulphid series. The crop was harvested when 60 days old. The results of this experiment are shown in Table X.

TABLE X.—*Effect of carbon bisulphid on the growth of buckwheat in different types of soil*

No.	Soil.	Carbon bisulphid added.	Weight of first crop.		
			Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*
1	Cecil clay	Control.	5	1.2	1.35
2	.....do	Control.	7	1.5	
3	.....do	2	12	3	3.5
4	.....do	2	15	4	
5	Porters clay	Control.	10.5	2	2.75
6	.....do	Control.	14.5	3.5	
7	.....do	2	14.5	3.7	3.15
8	.....do	2	10	2.7	
9	Clay loam	Control.	5.5	1.2	1.6
10	.....do	Control.	12.5	2	
11	.....do	2	28	6.5	7.25
12	.....do	2	30.5	8	
13	Hagerstown loam	Control.	27	6.2	5.6
14	.....do	Control.	25	5	
15	.....do	2	17.5	4.5	6
16	.....do	2	40.5	7.5	
17	Norfolk sand	Control.	32	8.7	9.45
18	.....do	Control.	49.5	10.2	
19	.....do	2	12	3	3.25
20	.....do	2	17.5	3.5	

With one exception, Norfolk sandy soil, the carbon-bisulphid series gave a larger yield. This was most marked in the case of clay-loam soil. The data on plant growth agreed with the plate counts.

The buckwheat was followed by a crop of Dwarf Essex rape. Unfortunately the young rape plants suffered seriously from insects. Although the tissue was too badly infested to save, a decided difference in growth could be seen. The beneficial effect of carbon bisulphid on rape was noted in every soil type.

EFFECT OF CARBON BISULPHID ON VARIOUS CROPS IN ACID SOILS

In order to study the effect of carbon bisulphid on the growth of higher plants in acid soils, a series of experiments was made. Four types of soil were selected for this work: Miami silt loam, Sparta sand, Colby silt loam, and Marshfield peat. The neutral Miami silt loam was used as a check for the acid soils. According to the Truog acidity test, Sparta sand requires 0.5227 gm. of calcium carbonate per 100 gm. of soil, Colby silt loam 1.021 gm., and Marshfield peat 4.43 gm. Four weeks after treatment with carbon bisulphid, the soils were planted.

RED CLOVER

The effect of carbon bisulphid on medium red clover in acid soils is clearly seen from the figures of Table XI. The clover grew luxuriantly in all soils except the untreated acid peat. Two crops were cut. Carbon bisulphid in peat soil caused an enormous gain in the growth of clover. This was very striking in both the first and second crop.

TABLE XI.—*Effect of carbon bisulphid on the growth of red clover in acid soils*

No.	Soil.	Carbon bisulphid added.	Weight of first crop, clover.			Weight of second crop, clover.		
			Green.	Dry.	Average green.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami silt loam	Control.	138		139	129	19	20
2	.....do	Control.	140	(a)		145	21	
3	.....do	2	158		141	168	26	23
4	.....do	2	124			131	20	
5	Sparta sand	Control.	36		34	58	13	11
6	.....do	Control.	33	(a)		48	10	
7	.....do	2	19		25	18	4	6
8	.....do	2	31			43	8	
9	Colby silt	Control.	95		91	110	20	17
10	.....do	Control.	87	(a)		85	14	
11	.....do	2	153		143	108	15	13
12	.....do	2	133			82	12	
13	Peat	Control.	4		3	6	2.8	2.4
14	.....do	Control.	2	(a)		5	2	
15	.....do	2	83		81	53	9	8.7
16	.....do	2	79			46	8.5	

[a] Lost.

Plate II, figure 2, shows the relative growth of clover in the treated and untreated soils.

Each figure for Miami silt loam in Table XI represents the average of triplicate jars. Because of the individual variation, it was decided to use 12 jars for this experiment. Six of these were used as controls and six treated with 2 per cent of carbon bisulphid. It is evident from the data that medium red clover in Miami soil is benefited both in the first and second crop by the antiseptic. In the Sparta sand a decrease was noted with each crop. The Colby silt loam gave a decided increase with the first crop, but not with the second.

Previous tests with these soils showed that the clover bacteria were present in sufficient numbers to produce good inoculation. In view of the large amount of carbon bisulphid applied, it was thought that this substance would probably injure nodule formation. However, examination of the root systems showed this was not the case. The plant roots were thoroughly inoculated, both in the treated and untreated soils. Apparently the plants in carbon bisulphid soils contained the greater number of nodules.

Because of the remarkable action of carbon bisulphid in peat soil, this part of the previous test was repeated. In addition to carbon bisulphid, the effect of flowers of sulphur was studied. If the data in the previous experiment are correct, the carbon bisulphid should greatly increase the growth of clover. A glance at the results in Table XII confirms this statement.

TABLE XII.—*Effect of carbon bisulphid and sulphur on the growth of red clover in peat soil*

No.	Soil.	Treatment.	Weight.		
			Green.	Dry.	Average.
			Gm.	*Gm.*	*Gm.*
1	Peat	Control	34	9	8.6
2	do	do	30	8.2	
3	do	1 per cent of carbon bisulphid.	95	21.5	21.7
4	do	do	110	22	
5	do	2 per cent of carbon bisulphid.	105	23	21.2
6	do	do	90	19.5	
7	do	0.3 per cent of sulphur.	8	3.5	2.2
8	do	do	4	1	

Carbon bisulphid causes a remarkable increase in the growth of clover on peat soil. There is apparently no decided difference in the action of 1 or 2 per cent of carbon bisulphid. Just why the volatile antiseptic should stimulate so markedly the growth of clover in the peat soil is not known. A more detailed study of the action of carbon bisulphid in peat is now under way. Flowers of sulphur at the rate of 0.3 per cent proved very injurious. In view of the high sulphur content of carbon bisulphid, it was thought that possibly free sulphur in peat might have somewhat the same effect.

CORN AND MUSTARD

The action of carbon bisulphid on corn and mustard in acid soils was studied in an experiment the results of which are given in Table XIII.

TABLE XIII.—*Effect of carbon bisulphid on the growth of corn and mustard in acid soils*

No.	Soil.	Carbon bisulphid added.	Weight of corn.			Weight of mustard.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
		Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami silt loam	Control.	190	50	64.5	83	18	19.5
2	do	Control.	360	79		145	21	
3	do	2	315	70	65	159	27	25.5
4	do	2	320	60		150	24	
5	Sparta sand	Control.	65	17	17.5	13	2.5	3
6	do	Control.	70	18		19	3.5	
7	do	2	100	21	23.5	11	2	2.5
8	do	2	120	26		12.5	3	
9	Colby silt	Control.	.....	83	84.5	.....	.....	10
10	do	Control.	393	86		67	10	
11	do	2	390	85	81	62	9.6	9.6
12	do	2	385	77		.....	.....	
13	Peat	Control.	160	28	24	0	.....	.....
14	do	Control.	130	20		0	.....	.....
15	do	2	165	25	22.5	0	.....	.....
16	do	2	.....	20		0	.....	.....

It is clear from the data that carbon bisulphid does not materially benefit corn. An exception to this was seen in the case of Sparta sand; in this instance the treated series showed a slight improvement.

A comparison of the growth of mustard in acid and in neutral soil shows that this crop grows best in a neutral soil. In Sparta sand and Colby silt loam the yield of mustard in the treated soil was below that of the control, while in the peat soil it failed entirely. It seems very probable that the acid reaction of the soil inhibits the growth of mustard. For instance, Kossovich (7) reports that mustard is sensitive to acidity. The addition of 2 per cent of carbon bisulphid to Miami soil stimulated the growth of mustard. This agrees with the results of previous tests. An increase in the growth of mustard has been noted in all four experiments with carbon bisulphid in Miami soil.

One series of jars, corn on Miami silt loam, was replanted to buckwheat. As previously reported, buckwheat showed a distinct improvement in the carbon-bisulphid soil. If the control weights are taken as 100, the treated series is equal to 115.

A review of all the data on the effect of carbon bisulphid on higher plants shows very clearly that carbon bisulphid does not produce the same effect on all plants. In almost every case (except acid soils) the carbon bisulphid favors in a decisive way the growth of mustard. Next in order of their response to carbon bisulphid come rape, red clover, buckwheat, oats, and corn. In acid soils, especially those rich in organic matter, the growth of clover is greatly favored by the carbon-bisulphid treatment.

The majority of the evidence indicates that carbon bisulphid is most beneficial to the growth of higher plants in peat or in open, sandy soils.

EFFECT OF CARBON BISULPHID ON THE GROWTH OF PLANTS IN SILICA SAND

If carbon bisulphid is a plant stimulant, then the addition of the proper amount to a nutrient solution for plants should exert a beneficial effect on the growth of higher plants. To test this a series of experiments was performed on different plants.

BUCKWHEAT AND OATS

Eight jars were filled with pure silica sand (99 per cent pure quartz), and the following ingredients added to each jar:

Water (H_2O)	500	c.c.
Potassium nitrate (KNO_3)	5	gm.
Ferrous phosphate ($Fe_3(PO_4)_2$)	1.25	gm.
Calcium phosphate ($Ca_3(PO_4)_2$)	1.25	gm.
Calcium sulphate ($CaSO_4$)	1.25	gm.
Magnesium sulphate ($MgSO_4$)	1.25	gm.

In addition to the soluble plant food, half of the jars received 2 per cent of carbon bisulphid. After treatment the jars were held for two months before planting to buckwheat and oats. The results of the test are given in Table XIV.

TABLE XIV.—*Effect of carbon bisulphid on the growth of buckwheat and oats in silica sand*

No.	Carbon bisulphid added.	Weight of buckwheat.			Weight of oats.		
		Green.	Dry.	Average.	Green.	Dry.	Average.
	Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Control.	15.8	3.2	2.3	3.5	1.5	1.3
2	Control.	8.5	1.4		3.4	1.2	
3	2	37.5	7	5.6	.....	.....	6.5
4	2	21	4.2		21	6.5	

It is apparent from the data that carbon bisulphid in silica sand exerts a beneficial effect on the growth of both buckwheat and oats. This agrees with the results of Koch (6)—that carbon bisulphid stimulates the higher plant growth. Although the duplicate jars do not agree very closely, the highest yield of the control was lower than any of the treated groups. For some unexplainable reason, the oats in jar 3 failed to grow. The young seedling died soon after germination. Plate II, figure 3, is a reproduction of a photograph of the buckwheat series.

CLOVER, BUCKWHEAT, AND MUSTARD

The foregoing experiment was repeated, using 3-kgm. jars and Tollen's medium. Only 1 per cent of carbon bisulphid was added. The jars were planted 30 days after treatment. The yields of the different crops are presented in Table XV. From the beginning clover and mustard began to show the favorable effect of carbon bisulphid.

TABLE XV.—*Effect of carbon bisulphid on the growth of buckwheat, clover, and mustard in silica sand*

No.	Carbon bisulphid added.	Weight of buckwheat.			Weight of clover.			Weight of mustard.		
		Green.	Dry.	Average.	Green.	Dry.	Average.	Green.	Dry.	Average.
	Per cent.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Control.	49	7.5	7	4.5	1	1.4	77	9	9
2	Control.	41	6.6		10	1.8		.....	.....	
3	1	49	7.8	7.6	12	2.2	2.2	52	5.8	8.6
4	1	45	7.5		12	2.3		98	11.5	

As compared with the results shown in Table XIV, the increase in the growth of buckwheat with carbon bisulphid was much smaller. The clover crop was about doubled in the presence of carbon bisulphid. Mus-

tard did not do well in sand cultures; growth was very irregular. Because of the size of the jars and the irregular growth of the crops it will be necessary to repeat the experiment.

EFFECT OF CARBON BISULPHID IN REINOCULATED SOIL

In the first part of this paper it has been shown that if soil treated with carbon bisulphid is reinoculated with fresh soil the bacterial processes are altered. The increase in number of bacteria attains a maximum much sooner and begins to decline earlier than in soil treated with carbon bisulphid but not reinoculated. This is also noted in the formation of soluble nitrogen. In order to record the effect on plant growth, the following experiment was planned. Six jars with 9 kgm. each of Miami silt-loam soil were used. Two months after treatment with carbon bisulphid, 2 per cent of untreated soil were added to jars 5 and 6. An equal amount was removed before the original soil was added. All of the jars were kept for another month before planting.

Plate counts three months from the date of treatment showed a decided increase in number of bacteria in the carbon-bisulphid soils. No appreciable difference existed between the carbon bisulphid and the carbon-bisulphid reinoculated soil.

The effect of treatment on nitrate content is evident from the following figures: If the nitrate nitrogen at the beginning is 100, then the control after three months is 370, carbon bisulphid is 50, and carbon bisulphid plus 2 per cent of the original soil is 44. Here, again, the inverse relation of number of bacteria and nitrate content is noted.

Protozoa were found in all of the soils and apparently in about the same number two months after treatment as in the original soil.

The effect of this treatment on the growth of oats and corn may be seen from the figures in Table XVI.

TABLE XVI.—*Effect of carbon bisulphid on the growth of oats and corn in reinoculated soil*

No.	Soil.	Treatment.	Weight of first crop, oats.			Weight of second crop, corn.		
			Green.	Dry.	Average.	Green.	Dry.	Average.
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	Miami.	Control.	168	51	50. 9	107	28	29
2	...do...	.....do.	178	50. 75		108	30	
3	...do...	2 per cent of carbon bisulphid.	178	50	52	86	33	29. 5
4	...do...	.....do.	185	54		83	26	
5	...do...	2 per cent carbon bisulphid plus 2 per cent of the original soil.	178	51. 5	56. 5	79	22	24
6	...do...	.....do.	215	61. 5		101	26	

The average dry weight of oats in soil treated with carbon bisulphid was slightly greater than that of the control. This difference was most noticeable in the case of reinoculated soil. It appears that the reinoculation benefits the action of carbon bisulphid on the growth of oats. The second crop of corn gave the opposite results. The corn in untreated soil gave the highest yield.

EFFECT OF CARBON BISULPHID ON THE ACCUMULATION OF SULPHATES IN SOIL

Very soon after the jars were planted it was observed that the surface of carbon-bisulphid soil was partly covered with needle-like crystals. Qualitative tests showed that these were made up largely of sulphates, possibly magnesium sulphate. The occurrence of salts was noted in several of the soils treated with carbon bisulphid. Possibly a part of the carbon bisulphid was oxidized to sulphates. It has been reported that a small portion of the carbon bisulphid may be converted into sulphates (4, p. 247–251; 10, p. 151–152).

Samples of the treated and untreated soils were analyzed for sulphates.[1] The results are shown in Table XVII.

TABLE XVII.—*Effect of carbon bisulphid on the accumulation of sulphates in the soil*

No.	Time.	Treatment.	Sulphur as sulphates.
	Months.		*Per cent.*
1	1	Untreated	0.023
2	1	2 per cent of carbon bisulphid	.038
3	3	Untreated	.018
4	3	2 per cent of carbon bisulphid	.039
5	5	Untreated	.019
6	5	2 per cent of carbon bisulphid	.060

It is apparent from the data in this table that the addition of carbon bisulphid tends to increase the sulphate content of the soil.

CONCLUSIONS

The addition of carbon bisulphid to soil exerts a decided effect on the fauna and flora of the soil. This is characterized by a temporary reduction in the number of micro-organisms. Later, an enormous multiplication of bacteria takes place and an almost parallel increase in production of by-products or soluble nitrogen is noted. The ammonia content seems to follow the curve of bacterial growth and later gives way to larger amounts of nitrate. From the evidence it seems that carbon bisulphid in soil produces an increase in soluble compounds of nitrogen and sulphur.

[1] The author is indebted to Prof. W. E. Tottingham, of the Department of Agricultural Chemistry, for the analyses.

In Miami soil carbon bisulphid benefited the growth of buckwheat, oats, and mustard. No relation seems to exist between plant stimulation with carbon bisulphid and the form of the soluble nitrogen. In non-acid soils carbon bisulphid is most beneficial to sulphur crops. Mustard offers a good example. In all of the experiments, except acid soils, mustard showed an increased growth from the use of carbon bisulphid. Carbon bisulphid in peat soil greatly benefits the growth of red clover. In sand cultures plus soluble plant food carbon bisulphid favors the growth of certain plants.

The data show clearly that carbon bisulphid does not act alike in all soils or toward all crops.

LITERATURE CITED

(1) FRED, E. B.
1911. Über die Beschleunigung der Lebenstätigkeit höherer und niederer Pflanzen durch kleine Giftmengen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 31, No. 5/10, p. 185–245, 4 fig. Literatur, p. 242–245.

(2) GAINEY, P. L.
1914. Effect of CS_2 and toluol upon nitrification. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 39, No. 23/25, p. 584–595, 2 fig. Literature, p. 595.

(3) GERLACH and VOGEL.
1905. Ammoniakstickstoff als Pflanzennährstoff. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 14, No. 3/4, p. 124–128, 2 fig.

(4) HEINZE, B.
1907. Einige weitere Mitteilungen über den Schwefelkohlenstoff und die CS_2-Behandlung des Bodens. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 18, No. 1/3, p. 56–74, 1 fig.; No. 7/9, p. 246–264, 2 fig.; No. 13/15, p. 462–470; No. 19/21, p. 624–634; No. 24/25, p. 790–798.

(5) HILTNER, Lorenz, and STÖRMER, Kurt.
1903. Studien über die Bakterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *In* Arb. Biol. Abt. Land- und Forstw. K. Gsndhtsamt., Bd. 3, Heft 5, p. 445–545, 4 fig., pl. 9–10.

(6) KOCH, Alfred.
1899. Untersuchungen über die Ursachen der Rebenmüdigkeit mit besonderer Berücksichtigung der Schwefelkohlenstoffbehandlung. Arb. Deut. Landw. Gesell., Heft 40, 44 p., 5 pl.

(7) KOSSOVICH, P. S., and ALTHAUSEN, L.
1909. Influence of $CaCO_3$ and $MgCO_3$ on the soil and plants. (Abstract). *In* Exp. Sta. Rec., v. 23, no. 3, p. 226. 1910. Original article appeared in Trudui Mendelyevsk. Syezda Obshch. i Prikl. Khim., god 1, 1907, p. 490–493. 1909. Not seen.

(8) KRÜGER, Wilhelm.
1905. Über die Bedeutung der Nitrifikation für die Kulturpflanzen. *In* Landw. Jahrb., Bd. 34, Heft 5, p. 761–782, pl. 13–15.

(9) LEHMANN, Jul.
1875. Ueber die zur Ernährung der Pflanzen geeignetste Form des Stickstoffes. *In* Centbl. Agr. Chem., Bd. 7, p. 403–409.

(10) MORITZ, J., and SCHERPE, R.
1904. Über die Bodenbehandlung mit Schwefelkohlenstoff und ihre Einwirkung auf das Pflanzenwachstum. *In* Arb. Biol. Abt. Land- u. Forstw. K. Gsndhtsamt., Bd. 4, Heft 2, p. 123–156.

(11) RUSSELL, E. J., and HUTCHINSON, H. B.
1909. The effect of partial sterilisation of soil on the production of plant food. *In* Jour. Agr. Sci., v. 3, pt. 2, p. 111–144, 4 fig., pl. 8–9.
(12) ——— ———
1913. The effect of partial sterilisation of soil on the production of plant food. II. The limitation of bacterial numbers in normal soils and its consequences. *In* Jour. Agr. Sci., v. 5, no. 2, p. 152–221, 7 fig.
(13) SHERMAN, J. M.
1914. The number and growth of protozoa in soil. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, No. 18/23, p. 625–630.

PLATE I

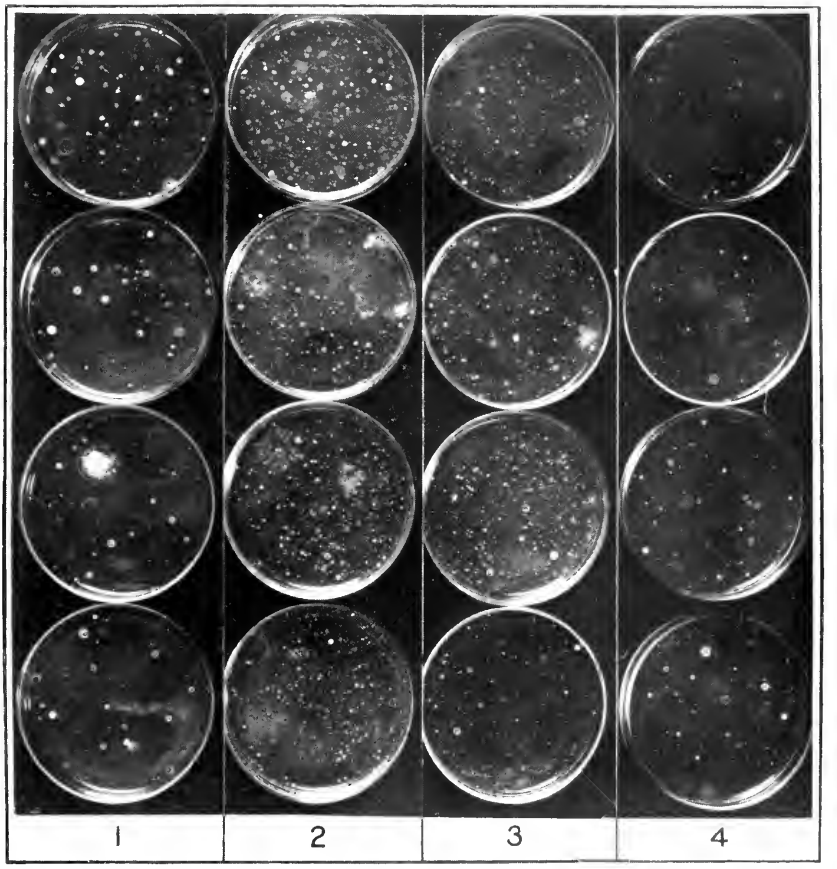
Plate cultures of soil organisms growing on agar:

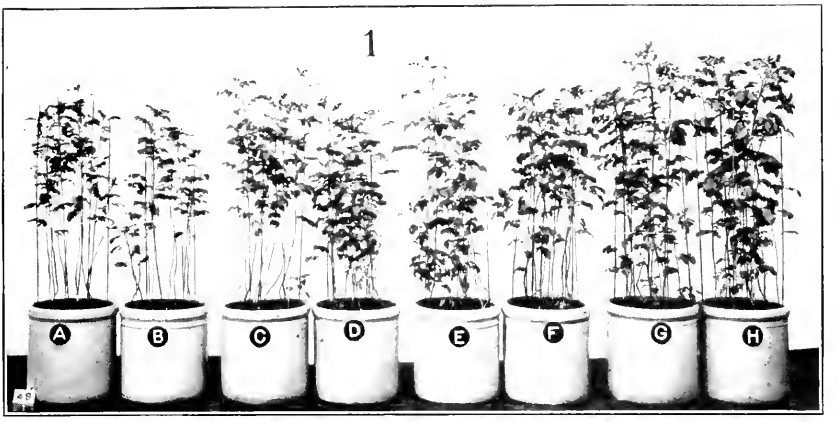
Fig. 1.—Colonies of organisms from untreated soil.

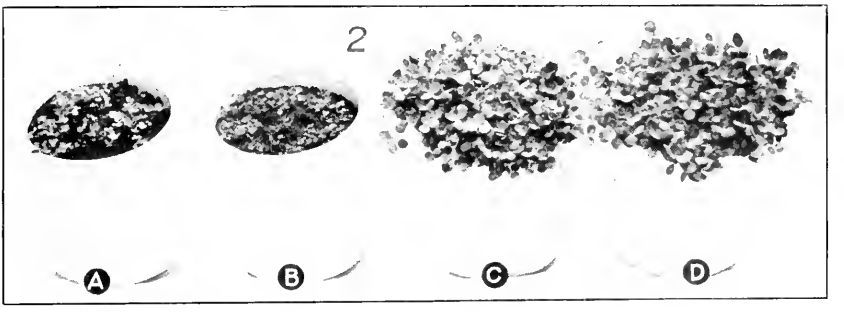
Fig. 2.—Colonies from soil treated with 2 per cent of carbon bisulphid.

Fig. 3.—Colonies from soil treated with 2 per cent of carbon bisulphid and evaporated.

Fig. 4.—Colonies from soil treated with 2 per cent of carbon bisulphid, evaporated, and reinoculated with 5 per cent of soil from an untreated jar.


1
2
3
4


1
A
B
C
D
E
F
G
H


2
A
B
C
D

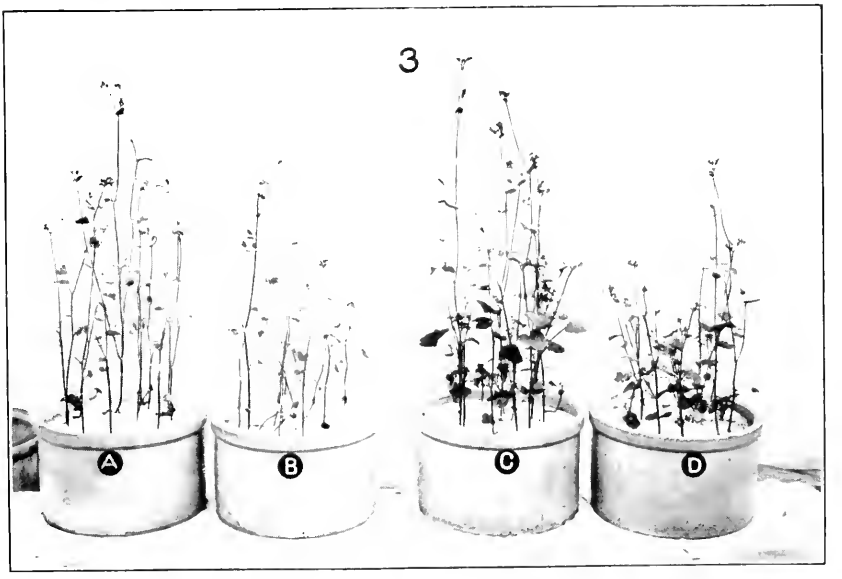

3
A
B
C
D

PLATE II

Fig. 1.—Effect of varying amounts of carbon bisulphid on mustard; *A*, *B*, soil untreated; *C*, *D*, soil treated with 0.5 per cent of carbon bisulphid; *E*, *F*, soil treated with 1 per cent of carbon bisulphid; *G*, *H*, soil treated with 2 per cent of carbon bisulphid.

Fig. 2.—Effect of carbon bisulphid on clover in peat soil; *A*, *B*, soil untreated; *C*, *D*, soil treated with 2 per cent of carbon bisulphid.

Fig. 3.—Effect of carbon bisulphid on buckwheat in sand cultures; *A*, *B*, soil untreated; *C*, *D*, soil treated with 2 per cent of carbon bisulphid.

CLIMATIC CONDITIONS AS RELATED TO CERCOSPORA BETICOLA [1]

By VENUS W. POOL, *Assistant Pathologist*, and M. B. MCKAY, *Scientific Assistant*, *Cotton and Truck Disease Investigations, Bureau of Plant Industry*

INTRODUCTION [2]

Climatic conditions of both winter and summer bear an important relation to the vitality and development of *Cercospora beticola*. During cold weather certain conditions enable the fungus to overwinter, while certain other conditions are inimical to its growth, a fact which has an important bearing on the control of the disease, as the earliest infections on growing sugar beets (*Beta vulgaris*) originate from the overwintered fungus. In the early summer, after infection occurs, temperature, relative humidity, rainfall, and wind directly affect the development of the fungus, the rapidity of conidial production, and subsequent infection.

OVERWINTERING

From the investigations here described it seems evident that under ordinary field conditions of winter the conidia of *C. beticola* usually live but a short time, although under ordinary herbarium conditions desiccation takes place only after exposure for several months. The sclerotia-like bodies (fig. 1, *A*, *a*), or masses of mycelium, the most resistant part of the fungus, which are embedded in the infected areas of the leaf blades and petioles, however, live over the winter under favorable conditions and in the spring produce conidia from the remnants of the old conidiophores (fig. 1, *A*, *b*), or both conidiophores and conidia (fig. 1, *A*, *c*) may be formed anew. For the purpose of making direct microscopical observation of such development sections of infected tissue which had been stored throughout the winter under favorable conditions were placed in hanging-drop cultures of bean agar. New conidiophores (fig. 1, *B*, *b*) grew from the masses of embedded mycelium, and although somewhat abnormal they produced rather typical conidia (fig. 1, *B*, *c*), thus showing that such material may be a source of early infection of growing plants.

[1] The investigations were carried on entirely in the field. Preliminary work was conducted during 1911 and 1912 at Rocky Ford, Colo. The detailed data were collected during 1912 and 1913 at Rocky Ford, which is in the Arkansas Valley of Colorado, a semiarid region under irrigation, and during 1914 near Madison, Wis., where the rainfall and average humidity were greater.

[2] The writers are indebted to Mrs. Nellie E. Fealy, of the Bureau of Plant Industry, for aid in editing and revising the manuscript.

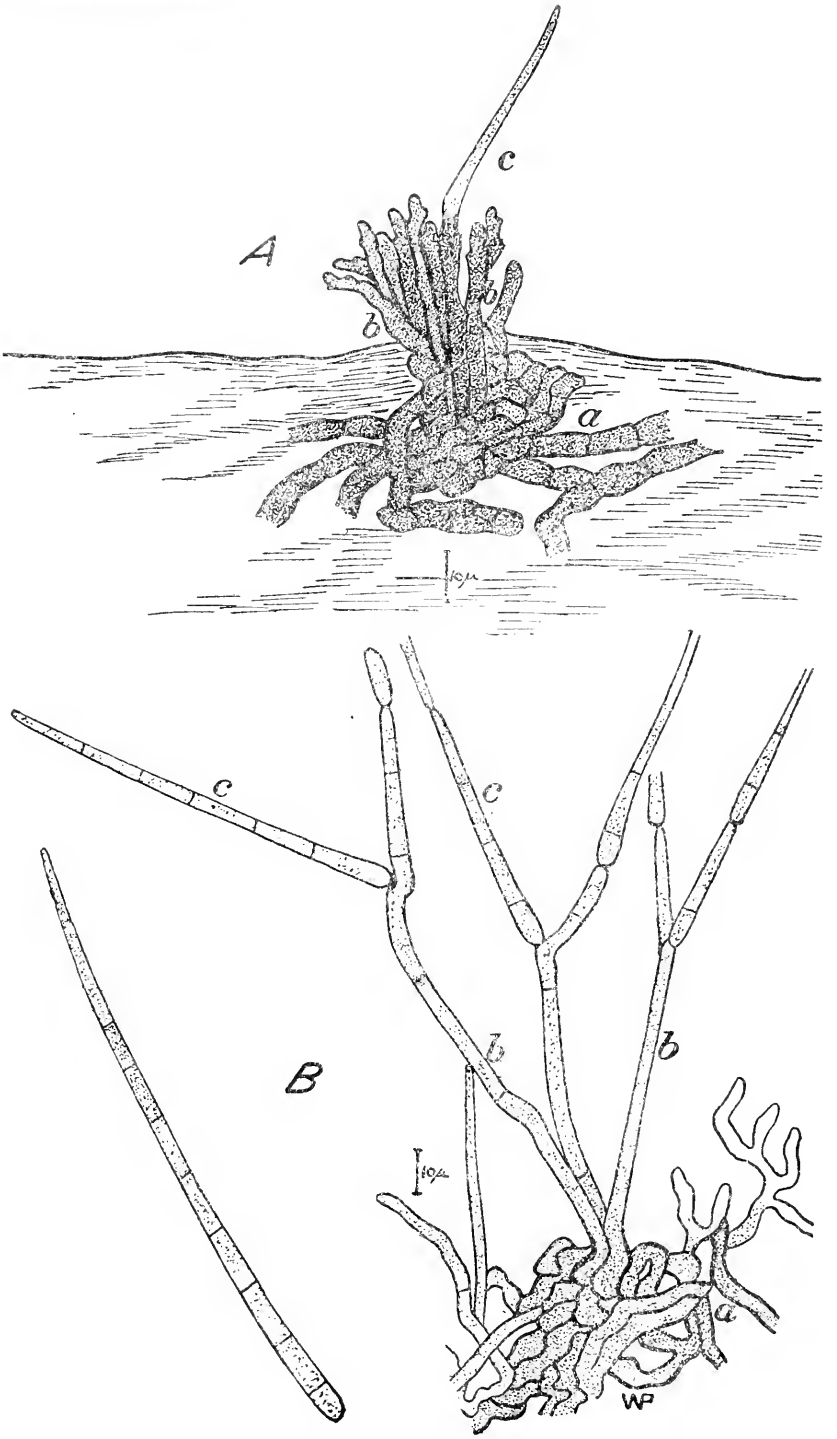


FIG. 1.—*Cercospora beticola*: *A*, Section of overwintered sugar-beet leaf showing embedded sclerotia-like body, *a*, with a mass of old conidiophores, *b*, from which a new conidium, *c*, was produced. *B*, Production of rather typical conidiophores, *b*, and conidia, *c*, from a sclerotia-like mass, *a*, taken from overwintered host material and placed in hanging-drop cultures.

CONIDIA

Thümen (1886, p. 50–54)[1] believed that the spores of *Cercospora beticola* are able to live for a certain length of time in the soil and retain their viability and produce new infection, and Pammel (1891, p. 238–243) and Massee (1906, p. 52–53) accord with this view. In the investigations here considered it was found that when kept dry, as in the case of herbarium material, the conidia remained viable for 8 months (Table I, tests 10 to 13), but soon after that no growth occurred. Only rarely were conidia found on the infected areas of the leaves which were exposed to outdoor weather conditions, and such conidia seemed to lose their vitality soon after harvest. No germination was found to take place under optimum conditions in the case of conidia which had been thus exposed from 1 to 4 months (tests 14 and 15). However, conidia occasionally found on spots that had been well protected, for instance in the interior of a pile of hayed beet tops, retained their viability for from 5 to less than 12 months (tests 16 and 17). Since the conidia are rarely found after a short time even on infected material that has been well protected and since they rarely germinate after being exposed outdoors for even 1 month after harvest, it would seem that under ordinary field conditions they play no important part in the overwintering of the fungus.

TABLE I.—*Viability of the conidia of Cercospora beticola as affected by desiccation*

Test No.	Environment.	Period of exposure.	Viability.
1	Stored, dry	14 years	None.
2	.....do	11 years	Do.
3	.....do	10 years	Do.
4	.....do	5 years	Do.
5	.....do	4 years	Do.
6	.....do	3 years	Do.
7	.....do	2 years	Do.
8	.....do	11 months	Do.
9	.....do	10 months	Do.
10	.....do	8 months	Slightly viable.
11	.....do	7 months	Extremely viable.
12	.....do	6 months	Do.
13	.....do	5 months	Do.
14	Left in field after harvest, Colorado	1 month	None.
15	Left in field after harvest, Wisconsin	4 months	Do.
16	Stored inside pile of hayed sugar-beet leaves	5 months	Extremely viable.
17	.....do	12 months	None.

SCLEROTIA AND MYCELIUM

Various investigators have attempted to determine whether different fungi live in the soil over winter and the manner in which they overwinter. Treboux (1914) found that the mycelia of several different rusts overwinter on host material freely exposed to climatic conditions. Stewart (1913) placed in boxes of soil potato leaves and tubers infected with *Phytophthora infestans*, exposed them to outdoor winter conditions, and found that plants grown on such soil developed no blight. However,

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 60.

temperature and moisture conditions in boxes of soil exposed aboveground to winter conditions are much more varied than in soil at different depths in the field where normal overwintering usually occurs.

In the overwintering experiments here described the host material was kept in an environment comparable to ordinary field conditions. The experiments at Rocky Ford, Colo., were started about the middle of October, 1912, and continued for 11 months. In these experiments some of the infected material was mixed with soil, placed in boxes, and exposed aboveground during the winter (Pl. III, 1); a second portion was buried from 1 to 8 inches in the ground (Pl. III, 2), wire netting being used above and below the infected material to insure ready location when examinations were made for cultural tests (Pool and McKay, 1915); a third portion of the infected tops was placed in a pile on top of the ground (Pl. III, 3). During the experiment records were kept of soil and air temperatures, the former being taken at a depth of 5 inches and the latter being obtained from the Weather Bureau station at Rocky Ford.[1]

The experiments carried on near Madison, Wis., were started the last of November, 1913, and continued through the winter. Infected sugar-beet tops were buried in the soil at depths of 5 and 8 inches, while seed-beet stalks were left under ordinary conditions in the field. In this experiment also records were kept of soil and air temperatures, the former being taken from March until June at a depth of 5 inches and the latter obtained from the Weather Bureau station at Madison.

The effect of desiccation on material kept under herbarium conditions was to kill probably all life of the fungus within 12 months, as already shown, but material kept under an environment having more or less moisture accompanied by the disintegrating action of various organisms was affected in an entirely different manner, as will be shown. All cultures from the infected material used in the two experiments above outlined were made from definite leaf-spots. Although the diseased tissue was the last to be completely disorganized and consequently could be found as long as any portion of the leaf remained, it became more and more difficult to obtain such tissue as time went on.

The fungus was unable to survive six months' outdoor exposure in boxes of soil (Table II, experiment 2), and this was also true of the fungus on leaves which had been freely exposed to outdoor conditions—for instance, on the outside of a hayed pile of sugar-beet tops (experiment 3), and on leaves buried 6, 7, and 8 inches in the ground (experiments 19 to 23). In cultures from infected mother-beet stalks and leaves that had been left in the field for a time and then plowed under or stored there was no growth, or only an indefinite growth, of the fungus after 7 months (experiments 8 to 10), while in infected material that had been protected in the interior of a pile of hayed beet tops (experiment 4) and in material

[1] All the records included in this paper from the Weather Bureau station at Rocky Ford, Colo., were kindly furnished by Mr. P. K. Blinn, the local observer.

that had been slightly covered or buried from 1 to 5 inches in the ground the life of the fungus was entirely extinct after 12 months (experiments 11 to 18). The death of the fungus in material plowed under is due in all probability to the rapid disorganization which results under favorable temperature and moisture conditions, such, for instance, as those which prevailed at Rocky Ford through the winter of 1912–13. During that period there was insufficient moisture to permit severe freezing, but there was a daily extreme variation of soil temperature, indicating that the air temperature produced the changes through the more or less dry soil. In the experiments at Madison there was only a partial disintegration of the buried beet tops six months after harvest, but other factors impaired the vitality of the fungus and its life appeared to be entirely extinct; consequently, notwithstanding the great differences in soil factors, comparable results as to the life of the fungus were obtained from the experiments at both places.

TABLE II.—*Effect of desiccation and overwintering on the viability of Cercospora beticola in infected sugar-beet tops under field conditions at Rocky Ford, Colo., and Madison, Wis.*

Experiment No.	Environment of sugar-beet-top material.	Period of exposure.	Number of spots from which cultures were made.	Number of viable spots.	Condition of leaves.
[a] 1	Dried, stored:				
	Illinois, Iowa	14 years	10	0	Good.
	Connecticut	11 years	10	0	Do.
	New York	10 years	10	0	Do.
	Wisconsin	5 years	10	0	Do.
	Iowa	4 years	10	0	Do.
	Maryland	3 years	10	0	Do.
	Colorado	2 years	10	0	Do.
	New Jersey	10 months	10	3	Do.
	Colorado	9 to 11 months	20	10	Do.
2	Stored in soil in boxes and left free under outdoor conditions, Colorado.	2 months	15	15	Do.
		3 months	7	7	Do.
		4 months	13	4	Do.
		5 months	12	2	Do.
		5½ months	12	0	Do.
		7 months	6	0	Do.
3	From the outside of "hayed" pile of sugar-beet tops, Colorado.	7 months	6	0	Do.
		10 months	13	0	Do.
4	From the interior of "hayed" pile of sugar-beet tops, Colorado.	2 months	10	10	Do.
		3 months	66	64	Do.
		4 months	29	27	Do.
		5 months	40	40	Do.
		7 months	18	12	Do.
		10 months	15	10	Do.
		12 months	25	0	Do.
5	In field, Colorado	2 months	10	10	Do.
		5 months	11	8	Do.
		8 months	10	2	Do.
6	Leaves from "mother beet" stalks free in field, Wisconsin.	5 months	21	15	Do.
7	First-year sugar-beet leaves free in field, Wisconsin.	5 months	32	3	Do.
		8 months	40	0	Partially disintegrated.
8	Spots on "mother beet" stalks free in field, Wisconsin.	4 months	7	[b] 7	Good.
		7 months	35	4?	Do.
9	Spots on "mother beet" stalks free in field 6 months, then plowed under 1 month, Wisconsin.	7 months	10	0	Somewhat softened.

[a] Herbarium specimens for this test were furnished by Barrett, Illinois; Clinton, Connecticut; Whetzel, New York; Pammel, Iowa; Norton, Maryland; and Cook, New Jersey.

[b] 395 colonies.

TABLE II.—*Effect of desiccation and overwintering on the viability of Cercospora beticola in infected sugar-beet tops under field conditions at Rocky Ford, Colo., and Madison, Wis.*—Continued

Experiment No.	Environment of beet-top material.	Period of exposure.	Number of spots from which cultures were made.	Number of viable spots.	Condition of leaves.
10	Spots on "mother beet" stalks free in field 4 months, then stored dry 3 months, Wisconsin.	7 months..	10	5?	Good.
11	Buried 1 inch in ground, Colorado....	6 months..	21	0	Partially disintegrated.
		7 months..	10	2	Do.
		10 months.	8	3	Greatly disintegrated.
		12 months.	24	0	Entirely disintegrated.
12	Buried 2 inches in ground, Colorado..	5 months..	14	2	Partially disintegrated.
		6 months..	19	10	Do.
		10 months.	11	0	Greatly disintegrated.
		12 months.	28	0	Entirely disintegrated.
13	Buried 3 inches in ground, Colorado..	6 months..	21	10	Partially disintegrated.
		10 months.	11	1	Greatly disintegrated.
		12 months.	18	0	Entirely disintegrated.
14	Broken and buried 3 inches in ground, Colorado.	6 months..	14	0	Greatly disintegrated.
		7 months..	12	5	Do.
		10 months.	16	0	Entirely disintegrated.
15	Buried 4 inches in ground, Colorado..	6 months..	19	5	Partially disintegrated.
		10 months.	19	1	Greatly disintegrated.
16	Buried 5 inches in ground, Colorado..	6 months..	20	4	Do.
		10 months.	15	0	Entirely disintegrated.
		12 months.	20	0	Do.
17	Buried 5 inches in ground, Wisconsin.	4 months..	30	0	Good.
		5 months..	30	3?	Do.
		6 months..	81	0	Do.
		7 months..	80	0	Partially disintegrated.
18	Plowed under in field, about 5 inches, Wisconsin.	5 months..	30	3?	Do.
		5 months..	20	0	Do.
19	Buried 6 inches in ground, Colorado..	5 months..	8	0	Greatly disintegrated.
		6 months..	20	4	Do.
		10 months.	12	0	Do.
		12 months.	15	0	Do.
20	Broken and buried 6 inches in ground, Colorado.	6 months..	18	0	Do.
		7 months..	10	0	Do.
		10 months.	16	0	Entirely disintegrated.
21	Buried 7 inches in ground, Colorado..	6 months..	15	0	Do.
		7 months..	10	0	Do.
		10 months.	12	0	Do
		12 months.	22	0	Do
22	Buried 8 inches in ground, Colorado..	6 months..	20	0	Do
		7 months..	10	0	Do.
		10 months.	12	0	Do.
23	Buried 8 inches in ground, Wisconsin.	4 months..	30	0	Good; ground frozen.
		5 months..	35	2?	Good.
		6 months..	57	0	Good; leaves have sour odor.
		6½ months	100	0	Partially disintegrated.

These experiments and observations made in the field during several spring and summer months showed that on leaves slightly protected on or near the surface of the ground during the winter *C. beticola* can live a sufficient length of time to be a source of infection for the succeeding sugar-beet crop and that the fungus is entirely killed by planting time when the infected material is plowed under to a depth of 6 to 8 inches in the fall.

AIR AND SOIL TEMPERATURES AT ROCKY FORD, COLO., AND AT MADISON, WIS.

Comparison of the air and soil temperatures which prevailed during the experiment at Rocky Ford and Madison showed a wide difference.

One of the most striking characteristics of these temperatures at Rocky Ford was the wide range between the maximum and the minimum, and this range may be observed throughout the entire records (fig. 2, 3). In the case of the soil temperatures especially, the wide range appeared to be due to a lack of moisture, the extreme variations being greater than if more moisture had been present. A comparison of the records shows that the variation in air temperature was much less and the mean daily temperature constantly lower at Madison than at Rocky Ford, notwithstanding the fact that the daily minimum temperature was usually lower at Rocky Ford. A comparison of the soil temperatures at the two points, however, shows that at Madison it probably remained more constant and was never as low as at Rocky Ford. This was due apparently to the greater amount of moisture in the soil at Madison and consequently its continued frozen condition. After March 23, the date on which the record was begun at Madison, the soil temperature at that place was never below 29° F., notwithstanding the fact that the air temperature was as low as 15° on April 8, while the minimum soil temperature at Rocky Ford was 22° on December 21 and 25° on February 8. However, as the air temperature on these dates was lower here than at Madison, comparisons can not be drawn too closely.

In view of the presence of snow on the ground, which, as is well known, protects the soil from the extreme variations of air temperature, and the prevailing low air temperatures, as shown by the records, it may be assumed that the soil temperatures at Madison during January and February and the early part of March varied but little from freezing. This assumption is supported by Frödin's experiments (1913), which showed in general that when the air temperature was much lower than that of the soil the soil temperature in ground covered with snow was higher than in bare ground. He found that temperatures taken at a depth of 10 cm. in the former were the same as those taken at a depth of 27.4 cm. in the latter. After the early part of April the minimum soil temperatures at Rocky Ford and Madison agreed closely, although the minimum air temperature at the former place remained generally the lowest of the temperatures recorded.

Temperatures obtained from the interior of a pile of hayed sugar-beet leaves by means of a soil thermograph buried in the pile varied less than temperatures taken outside the pile, as shown by the following records made on May 8, 1913, and as was probably the case during the entire winter season: Temperature inside pile, maximum, 67° F.; minimum, 58°; difference, 9°. Temperature outside pile, maximum, 84° F.; minimum, 45°; difference, 39°.

In view of the fact that the fungus lived twice as long inside the pile as it did on the outside it would seem that a more uniform temperature might be regarded as one of the controlling factors in the life of the fungus.

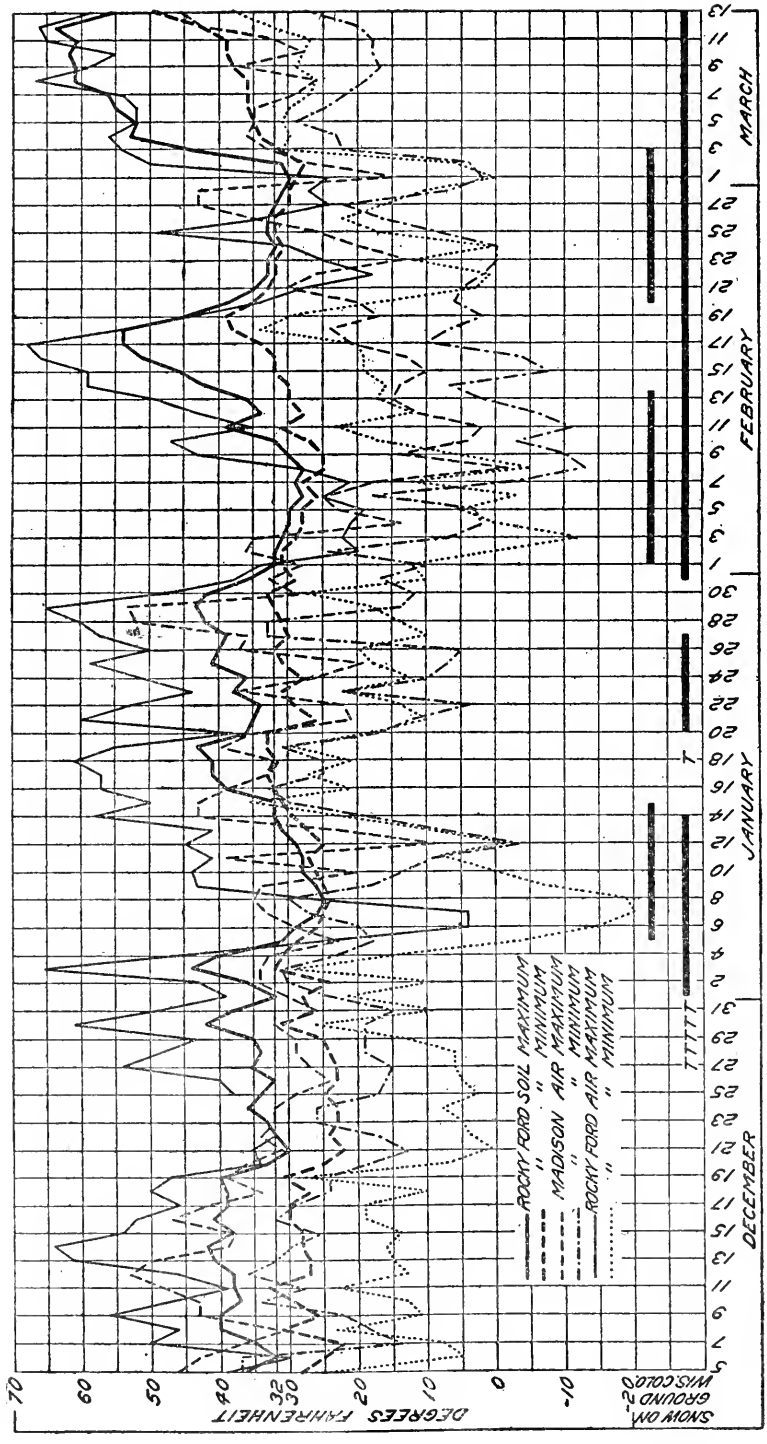


FIG. 2.—Curves of the maximum and minimum soil and air temperatures for the period from December 5, 1912, to March 13, 1913, at Rocky Ford, Colo., and air temperatures from December 5, 1913, to March 13, 1914, at Madison, Wis., together with the periods that snow covered the ground.

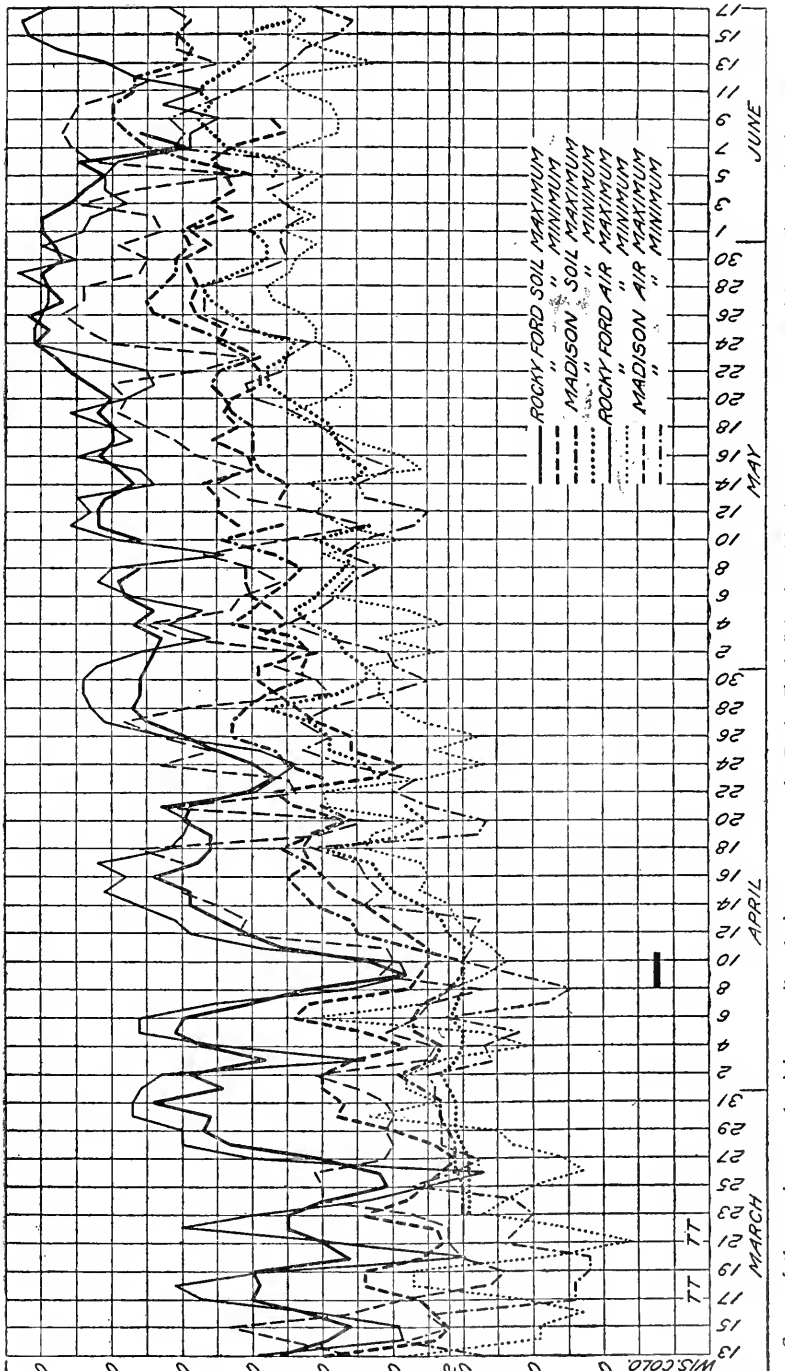


FIG. 3.—Curves of the maximum and minimum soil and air temperatures for Rocky Ford, Colo., from March 13 to June 17, 1913, and for Madison, Wis., from March 13 to June 17 1914

Low temperatures are not entirely inhibitive, as was shown by thermal tests of artificial cultures. After such cultures had been exposed to temperatures averaging 0.9° C. for 48 days and then kept at 28° C., numerous colonies developed. Also, heavily infected leaves kept at 0.9° C. for 97 days yielded good growth when cultures were made and held at favorable temperatures. Had the cultures been exposed to freezing temperatures or to extreme variations in temperature, the effect would doubtless have been more pronounced.

Although the temperature variations and the amount of soil moisture at Rocky Ford and Madison differed greatly, the effect on the life of the fungus was apparently the same at both places. It may be concluded that conditions of the soil which favor the process of disintegration are the most important factors in the control of the disease, and these experiments indicate that these processes are most active at a depth of 6 to 8 inches.

SUMMER CLIMATIC CONDITIONS

The summer climatic conditions here considered were recorded during 1913 in fields of first-year sugar beets grown at Rocky Ford, these fields being an example of the usual progress of the disease where neither rotation nor sanitation at the preceding harvest time had been practiced.

A study of the temperature and humidity records taken at different places in a beet field at Rocky Ford and at the Weather Bureau station 3 miles from the field was made to determine their comparative values in making important correlations. The records made in the sugar-beet field were taken by means of hydrothermographs kept in meteorological instrument shelters 5 feet above the ground (Pl. IV, fig. 1) and among the plants (Pl. IV, fig. 2). These were checked at frequent intervals with a sling and cog psychrometer (Shaw, 1914), respectively, and under Colorado conditions were found to be accurate. The records of the Weather Bureau station were taken by means of maximum and minimum thermometers kept in an instrument case about 5 feet above the ground in an open space (fig. 3).

The daily maximum and minimum temperatures and humidities, together with the total number of hours the humidity was above 60 from noon of the preceding day to noon of the given day, are used in the present interpretations. It has been found that when a high relative humidity prevails, the stomata of the sugar-beet leaves are usually open; and as the fungus enters the leaves only through the open stomata, the length of time they remain open is a fundamental factor in determining the possible occurrence of infection (Pool and McKay, 1916).

AIR TEMPERATURE AND RELATIVE HUMIDITY

The temperature and relative humidity taken with hygrothermographs placed near the ground among the plants varied widely from those taken with hygrothermographs in the air above the field and also from those

taken at the Weather Bureau station; hence, the place where the records were taken for use in the present correlations with the development of the disease is an important consideration.

AIR TEMPERATURE.—At Rocky Ford the maximum temperatures taken among the plants near the surface of the ground from June 13 to 30 ranged from 2 to 19 degrees higher and the minimum temperatures generally from 1 to 14 degrees lower than those taken at 5 feet above the ground (fig. 4). This was due to the fact that the plants were small during this period and covered only a portion of the ground; consequently during the daytime the temperature of the soil became higher than that of the air, and in turn the temperature of the air near the ground became higher than that of the air a few feet above. During the night the reverse occurred, the surface soil losing its heat by radiation and conduction faster and finally reaching a lower temperature than that of the air in contact with it, after which the heat of the latter gradually passed into the soil and as a result the temperature of the air immediately above the ground eventually became lower than that a few feet higher up. It is possible that convection currents also tended to lower the temperature of the air immediately above the ground; for, as is well known, when it is not disturbed by other factors, the coolest air settles to the lowest levels.

The maximum temperatures of the air near the ground, as shown by the records, were higher for a longer period during June than at any time during the season, varying from 100° to 106° F. on nine different days between the 14th and 26th of that month and rising above 100° only once thereafter, on August 16. The maximum temperature of the air 5 feet above the ground, on the other hand, was lower during June than during the middle of the season, ranging from 90° to 93° on six different days during the month, while it was above 90° and sometimes as high as 100° on 12 different days during July.

As shown by the records, the temperature of the air near the ground among the plants was lower during the middle than during the early part of the season. This was probably due to the difference in the size of the plants, the larger plants practically covering the ground in midseason and preventing the heating of the surface soil, while early in the season the smaller plants covered the ground but sparsely and consequently afforded less protection against heating. Comparison of the records also shows that during the middle of the season the temperature of the air among the plants near the ground was practically the same as that of the air 5 feet above the field and that throughout the entire period the latter was quite comparable with the temperatures taken at the Weather Bureau station (fig. 4).

A similar marked variation was shown at Madison, the maximum temperature there being almost constantly higher and the minimum tempera-

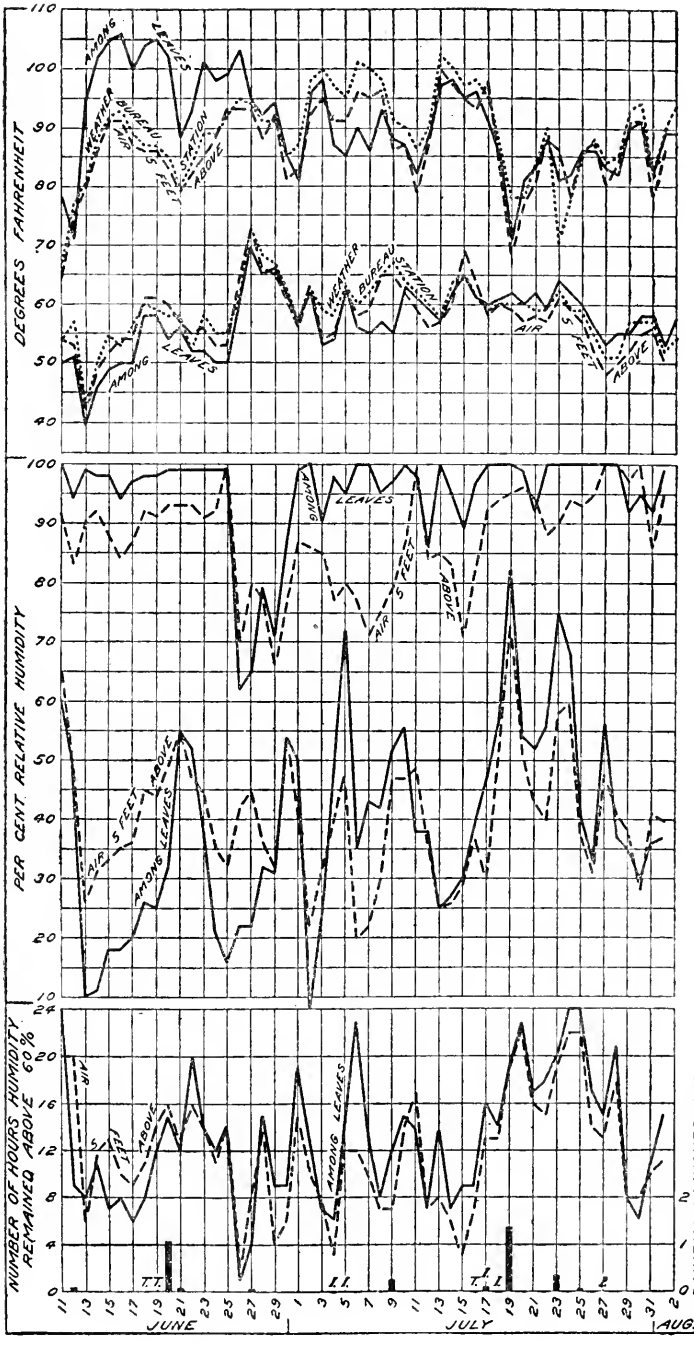


FIG. 4.—Curves of the maximum and minimum temperatures and relative humidities and the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among sugar-beet plants and in the air 5 feet above the field, together with the field rainfall and irrigation records. For comparison, the maximum and minimum temperature records from the Weather Bureau station are included. June 11 to August 2, 1913, at Rocky Ford, Colo.

ture usually lower among the beet plants than the temperature shown by the Weather Bureau records, which were taken on top of a four-story building about a mile from the sugar-beet field. These wide variations between the air temperature taken near the ground among the plants and that taken 5 feet above the field and between the former and the temperature taken at the Weather Bureau stations show that for correlation with fungous activities only the records taken among the plants should be used.

RELATIVE HUMIDITY.—There was also a wide variation in the humidity near the ground among the plants and 5 feet above the field. For instance, the daily minimum humidity at Rocky Ford from June 13 to 29, with two exceptions, was higher and remained above 60 generally for a longer period in the air above the plants than among the leaves near the ground (fig. 4), owing to the higher temperature at the surface of the ground as a result of the small amount of covering afforded by the young plants. During this period the daily variation of humidity among the leaves was extreme, ranging from 99 to 10 on June 13, from 99 to 16 on June 25, and from 100 to 8 on July 2. After June 29, on the other hand, the minimum humidity was generally higher, the humidity remained above 60 for a longer time among the leaves than in the air above, and the daily variation among the leaves was less extreme than earlier in the season. These conditions were due mainly to the greater amount of covering afforded by the larger plants and consequent longer retention of moisture among the leaves. The humidity both among the plants and in the air 5 feet above the field remained, on an average, above 60 for a longer time each day during midsummer than during June, owing in part to the increased use of irrigation water as the season advanced and the increased amount of moisture in the surrounding air resulting from the increased transpiration of the larger plants.

Comparison of the Madison and the Rocky Ford records (fig. 5) of the number of hours that the relative humidity remained above 60 each day among the sugar-beet plants shows that throughout the season it was higher, on an average, at Madison. Here it remained above 60 for a longer time each day during the latter half of June, when the records were started, and for a shorter time each day during August than during any other summer month. This was due to difference in the amount of rainfall, there being frequent rains during the former period and comparatively dry weather during the latter. At Rocky Ford the facts were reversed, the humidity remaining above 60 for a longer time each day during midseason than during the latter half of June or the first part of September. This was probably due to more frequent irrigation and the increased covering afforded by the larger plants of midseason.

Table III shows the average number of hours a day that the relative humidity remained above 60 at Madison and at Rocky Ford.

TABLE III.—*Average number of hours a day that the relative humidity was above 60 at Madison, Wis., and Rocky Ford, Colo., during the summer of 1914 and 1913, respectively*

Date.	Madison, Wis. (1914).	Rocky Ford, Colo. (1913).
	Hours.	*Hours.*
June 16 to 30	19.4	10.8
July	17.3	14.2
August	16.6	14.1
September 1 to 6	18.0	11.3
Seasonal average	17.4	13.4

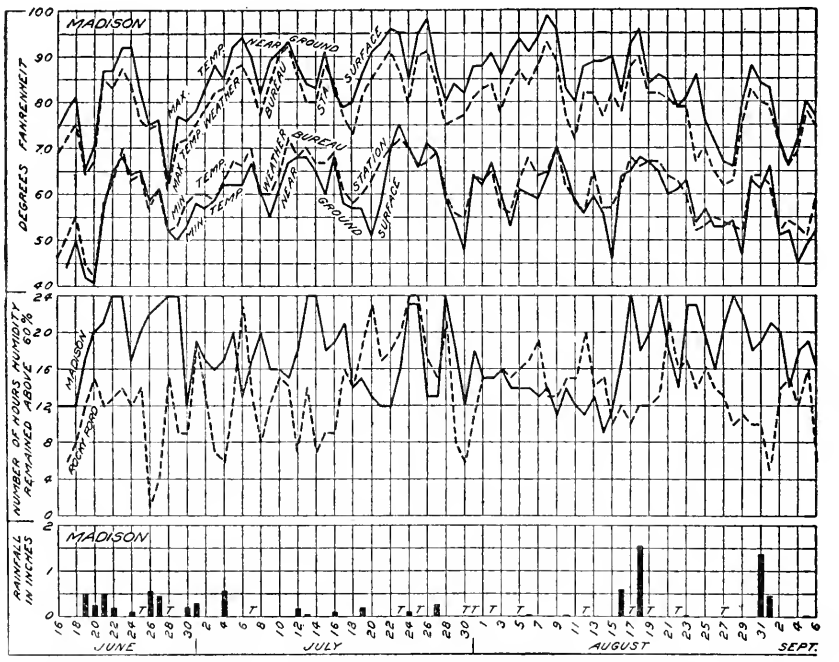


FIG. 5.—Curves of the maximum and minimum temperatures among sugar-beet plants and at the Weather Bureau station, and the seasonal rainfall records at Madison, Wis., in 1914, and the number of hours that the humidity remained above 60 among the sugar-beet plants in the field at Madison, Wis., in 1914, and at Rocky Ford, Colo., in 1913.

The greater average number of hours of high humidity at Madison accounts for the periods of extreme infection which occurred there when the fungus was present. Here leaves badly infected with *Cercospora beticola* and entirely covered with conidia were found at times, but this condition was rarely seen at Rocky Ford. There were numerous cases of cotyledon infections also at Madison, the high humidity early in the season favoring their occurrence; but no such infections were found at Rocky Ford.

RAINFALL AND IRRIGATION

The rainfall records made during the summer season of 1913 in the beet field at Rocky Ford in which infection was studied in detail (fig. 4, 7) where obtained by means of a rain gauge placed at the edge of the sugar-beet field (Pl. IV, fig. 1). Most of the rain was in the form of local showers, the amount varying greatly within a radius of less than 2 miles; but occasionally general rains fell. The effect of the increased relative humidity resulting from rainfall usually lasted longer among the leaves than in the air 5 feet above (fig. 7).

The effect of irrigation on humidity was found to be similar to the effect of rain. On July 2, before the field was irrigated, its humidity was as low as 8 and on July 3 and 4 remained above 60 for 7 and 6 hours, respectively. On July 4 and 5 the field was irrigated and the humidity remained above 72 on the 4th and above 60 during 23 hours of the 5th. On July 27 the field was again irrigated and the humidity remained above 60 for 15 hours that day and 21 hours the following day. On August 19 and 20 the field was irrigated the third time and the humidity remained above 60 for 12 and 13 hours, respectively, and the next day 21 hours. The general humid conditions necessary for leafspot infection, however, are developed much better by rain than by irrigation, because of the latter being comparatively local and unaccompanied by the atmospheric conditions attending rainfall.

WIND

Records of wind velocity at Rocky Ford were taken by means of an anemometer placed 6 feet in the air at the edge of the beet field (Pl. IV, fig. 1), the readings being made at irregular intervals and the velocities computed being the hourly averages from one reading to the next. As the records were not made daily, accurate hourly velocities for different intervals during the day can not be obtained from the records. They show, however, that the average seasonal velocity from June 12 to September 22 was 5.3 miles per hour. Occasionally two daily readings were made, one in the morning and the second late in the afternoon. These show that the average velocity of the wind was always higher during the day than at night, the greatest velocity usually prevailing in the afternoon during the period of lowest humidity. While no general dissemination of conidia was correlated with high wind velocity, the afternoon combination of highest wind with lowest humidity apparently favored the dissemination of conidia. In fact, in the case of air cultures made at different times during several days, it was found that the fungus grew usually only on those exposed during the afternoon.

SUMMER INFECTION CYCLES

The thermal relations of the fungus are closely linked with the effect of various climatic factors on the production and dissemination of conidia and on infection cycles. With a view to determining these relations the fungus was grown in Petri-dish cultures in thermostats at different and varied temperatures. At first the moisture was probably more or less constant, but as time went on it became relatively low. The effect of different temperatures, however, was comparable with that observed under existing field conditions.

THERMAL RELATIONS OF THE FUNGUS IN CULTURES

Tests of the fungus on string-bean agar were made at Washington during November and December, 1913, and January, 1914. The cultures were obtained from isolations made at the time of the tests from infected sugar-beet leaves collected at Rocky Ford during the preceding September. One colony of the first isolations was macerated in 10 c. c. of sterile water, and one platinum loop of this suspension was used for each tube of medium. Three poured plates were used for each single test. The cultures were exposed to different constant temperatures and to varied constant temperatures (high and low changed to low and high, respectively). Exposures were also made for 8 hours at the higher temperatures and then for 16 hours at lower temperatures, and, after a short interval of exposure in a certain number of these tests, both temperatures were lowered, it being possible in this way to approximate night and day temperatures in the field under normal conditions.

SERIES A (DIFFERENT CONSTANT TEMPERATURES).—When the cultures were held at different constant temperatures, the abundance and size of the individual colonies gradually increased, while the time necessary for development decreased with the temperatures 12.5°, 17.3°, 19.2°, 20°, and 30.8° C. The best growth was made at a temperature of 30.8°, but this in all probability was slightly above the optimum constant temperature, as no growth took place in cultures held for 9 days at 34.7°, 35.8°, and 40.6°, respectively (Table III, series A).

SERIES B (VARIED CONSTANT TEMPERATURES).—Although no growth of the fungus took place in cultures held at constant temperatures of 34.7° and 35.5° C., a small percentage of normal colonies developed in cultures exposed for three days to these temperatures and then for several days to a temperature of 30.8°, while in cultures exposed for three days to 40.5° no growth occurred when subsequently held at 30.8°. On the other hand, in cultures exposed for three days to a temperature of 30.8° there was almost a normal development of the colonies for three days after they were exposed to 34.7°, but at the end of five days the inhibitive effect of the latter temperature became manifest. In the case of cultures

changed from 30.8° to 40.5° only a very slight increase in growth was apparent during the first three days at the higher temperature, and after that it ceased entirely (Table III, series B).

TABLE III.—*Comparative diameter of colony growth (in millimeters) of Cercospora beticola at different constant temperatures,[a] at decreasing and increasing constant temperatures, and at daily varied temperatures*

SERIES A, CONSTANT TEMPERATURES

	Diameter of colony growth at temperature (°C.) of—										
Maximum	2.1	7.9	11.1	14.4	19.1	20.2	21.0	33.0	36.2	37.4	41.0
Minimum	0.3	4.0	8.0	11.5	16.3	18.6	19.5	29.7	33.8	34.5	40.0
Average	0.9	5.4	8.9	12.5	17.3	19.2	20.0	30.8	34.7	35.8	40.6
Period of growth:											
3 days	0	0	0	0	0.25	0.65	0.67	3.6	0	0	0
6 days	0	0	0	.25	.8	2.2	2.3	6.4	0	0	0
9 days	0	0	.25	.6	1.5	5	4.6	7	[b] 0	[b] 0	[b] 0
11 days	0	0	.4	.85	2.8	7	6.4	.....	.....	.....	.....
14 days	0	0	.5	1.5	4.2	.....	.....	.....	.....	.....	.....
18 days	0	0	.7	2.2	.....	.....	.....	.....	.....	.....	.....
22 days	0	0	1.2	3	.....	.....	.....	.....	.....	.....	.....

[a] The temperatures of each thermostat for all tests were averaged from two daily readings continued throughout the time of the experiment.
[b] No growth occurred in these plates when held at 28° C. for 10 days.

SERIES B, DECREASING AND INCREASING CONSTANT TEMPERATURES.

	Diameter of colony growth at temperature (°C.) of—				
	[a] 34.7	[a] 35.5	[a] 40.5	[b] 30.8	[c] 30.8
Period of growth:					
3 days	0	0	0	3.6	3.4
6 days	4	3.6	0	6	3.7
8 days	[d] 8.3	[e] 9.3	0	6.4	3.7

[a] Temperature changed to 30.8° C. after three days.
[b] Temperature changed to 34.7° C. after three days.
[c] Temperature changed to 40.5° C. after three days.
[d] Only 19.2 per cent of the normal number of colonies developed.
[e] Only 12.8 per cent of the normal number of colonies developed.

SERIES C, DAILY VARIED TEMPERATURES

	Diameter of colony growth at temperature (°C.) of—						
16 hours at	14.5	14.5	14.5	20	20	20	20
8 hours at	19.2	21.6	28	30.8	34.7	35.8	40.6
Period of growth:							
3 days	0.4	0.5	0.7	1.7	0.6	0.6	0
5 days	.7	1.9	3.4	6	3.6	3.2	0
7 days	2.7	3.6	5.2	8.8	4.8	5.4	0
9 days	.....	.....	.....	.....	.....	.....	0

SERIES C (DAILY VARIED TEMPERATURES).—In these tests the temperatures were made to correspond closely with summer outdoor temperatures of night and day by holding the cultures for 16 hours at the lower and for 8 hours at the higher. After seven days' exposure the growth of colonies

on cultures exposed to temperatures of 14.5° and 19.2° C. averaged 2.7 mm. in diameter; after exposure for the same length of time to 14.5° and 21.6°, 14.5° and 28°, and 14.5° and 30.8° the growth gradually increased until it reached a maximum diameter of 8.8 mm; but when exposed to higher temperatures (20° and 34.7° or 20° and 35.8°) the growth gradually diminished until finally it equaled approximately that attained under 14.5° and 28°. There was no growth on cultures exposed for nine days or longer to 20° and 40.6° (Table III, series C).

SERIES D (HIGH VARIED CHANGED TO LOW VARIED TEMPERATURES).—A plate culture exposed for three days to temperatures of 20° and 40.5° C., being held 16 hours at the lower and 8 hours at the higher, and then for six days at 20° and 30.6°, developed 23 colonies, averaging 10.3 mm. by the end of the latter period, while a check plate exposed constantly to a temperature of 30.6° developed 100 colonies by the end of the latter period. A plate exposed to the higher temperatures—20° and 40.5°—for five days and then held at 20° and 30.6° for six days developed six colonies at the end of the latter period, while a plate exposed to 20° and 40.5° and then held at 20° and 30.6° for seven days developed no growth of the fungus.

Later on in this paper the fact that high minimum and maximum temperatures inhibit the growth of the fungus, as brought out by these tests, is correlated with the effect of existing high field temperatures, with their consequent accompanying factors, on the leaf spot. Although the optimum temperature variations—20° and 30.8° C.—were found to be very favorable to the development of leafspot in the field, little or no increase in the disease was observed to follow high night and day field temperatures—20° and 40.5°, respectively.

It was also observed that different temperatures affect conidial septation. The normal average septation varies from 6 to 11, but during warm, humid periods the conidia were usually found to be many septate, sometimes as high as 20-septate, while after a cooler period, such as usually occurs in September, they were only from 2- to 4-septate.

RELATION OF CONIDIAL PRODUCTION AND DISSEMINATION TO CLIMATIC CONDITIONS

For the purpose of studying the relation of temperature and relative humidity to the production and dissemination of conidia, detailed life histories of a large number of individual spots on 10 plants in the medium-early field at Rocky Ford were kept during the season of 1913. The temperature and humidity records used in these correlations were those taken among the beet leaves near the ground and, together with rainfall and dates of irrigation, are shown in figure 7.

Beginning with the outermost or oldest, the leaves were tagged and numbered consecutively, and the location of the spots on each was indi-

cated on diagrams. As new leaves developed, they were included in the observations, and this was true also of new spots, until they became too numerous, after which only a few representative ones on each leaf were studied in detail. During the period from the 24th of June to the 19th of September 330 spots were studied, both surfaces being examined at frequent intervals with a hand lens. For the purpose of getting a basis for comparison of rates of development at different stages in the life history of the disease, percentage values were assigned to each stage as follows, the spots being grouped and averaged later (Table IV):

Percentage value.	Stage of development of fungus.
5.0	Spot first noticed. Neither conidia nor conidiophores present.
12.5	Conidiophores present.
19.7	Very few conidia.
25	Few conidia.
31.2	Conidia fairly numerous.
37.5	Conidia numerous.
43.7	Conidia fairly abundant.
50	Conidia abundant.

The value of the spot is the sum of the values of the two sides—that is the value of a spot on which there were but few conidia (25) on one side, and abundant conidia (50) on the other, is 75. Again, the value of a spot on which conidiophores only (12.5) were present on one side, and very few conidia (19.7) on the other, is 32.[a]

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*[b]

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
**2	3	5	July 7	10	No conidiophores on either surface. July 8, no change.
			10	37	Conidiophores above, conidia few below.
			12	50	Conidia few on both surfaces. July 14, no change.
			16	50	Conidia very few above, fairly numerous below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	62	Conidia fairly numerous on both surfaces.
			28	37	Conidia none above and few below.
2	4	1	2	10	No conidiophores on either surface. July 7, no change.
			8	24	Conidiophores on both surfaces.
			10	69	Conidia very few above and abundant below.
			12	75	Conidia few above and abundant below.
			14	100	Conidia abundant on both surfaces. July 16, 21, 23 (leaf yellow), July 25, no change.
			28	63	Conidia numerous above and few below.
2	4	3	7	10	No conidiophores on either surface. July 8, no change.
			10	25	Conidiophores on both surfaces.
			12	62	Conidia fairly numerous on both surfaces.
			14	82	Conidia numerous above and fairly abundant below.
			21	100	Conidia abundant on both surfaces.
2	4	4	7	10	No conidiophores on either surface.
			8	25	Conidiophores on both surfaces.
			10	75	Conidia few above and very abundant below. July 14, no change.
			16	57	Conidia very few above, numerous below.

[a] For convenience the decimal fractions, which make only a negligible difference in the averages, are omitted.

[b] In Table IV asterisks (*), daggers (†), and section marks (§) are used to designate definite leaf spots to which reference is made in the text.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
**2	6	1	July 7	15	Conidiophores forming above, nothing below.
			8	31	Conidiophores above, conidia very few below.
			10	100	Heavy production of conidia on both surfaces. July 12, 14, 16, 21, 23, no change.
			25	63	Conidia few above and numerous below.
3	2	1	June 24	10	No conidiophores on either surface. June 25, 26, 27, 28, 30, July 1, 2, 7 (leaf yellow), 8, no change.
3	2	2	25	10	No conidiophores on either surface. June 25, 26, 27, 28, 30, July 1, 2, 7, 8, no change.
3	4	1	July 7	10	No conidiophores on either surface.
			8	15	Conidiophores forming only on lower surface.
			10	62	Conidiophores abundant above and conidia abundant below.
			12	100	Conidia abundant on both surfaces. July 14, no change.
			16	69	Conidia very few above and abundant below.
			21	25	No conidia on either surface.
**3	8	1	7	10	No conidiophores on either surface. July 8, no change.
			10	25	Conidiophores abundant on both surfaces.
			12	100	Conidia abundant on both surfaces. July 14, 16, 21 (center of spot gone), no change.
			23	94	Conidia fairly abundant above and abundant below.
			25	50	Conidia few on both surfaces, leaving and leaf yellowing.
**3	8	2	7	10	No conidiophores on either surface. July 8, no change.
			10	25	Conidiophores abundant on both surfaces.
			12	100	Conidia abundant on both surfaces. July 14, no change.
			16	94	Conidia fairly abundant above and abundant below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	50	Conidia few on both surfaces.
†3	8	3	7	10	No conidiophores on either surface. July 8, no change.
			10	25	Conidiophores abundant on both surfaces.
			12	100	Conidia abundant on both surfaces. July 14, no change.
			16	94	Conidia fairly abundant above and abundant below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	75	Conidia few above and abundant below.
3	9	1	23	20	Conidiophores numerous above and few below.
			25	44	Conidia few above and very few below.
			28	50	Conidia few and matted together on both surfaces.
4	1	1	June 24	10	No conidiophores on either surface. June 25 (leaf dying), 26 (leaf dead), 27, 28, 30, July 1, no change.
4	3	1	July 8	10	No conidiophores on either surface.
			12	62	Conidia fairly numerous on both surfaces.
			14	50	Conidia leaving, few on both surfaces. July 16, 21 (leaf dead), no change.
**4	5	1	June 30	10	No conidiophores on either surface. July 1, 2, no change.
			July 7	30	Conidia forming on both surfaces. July 8, no change.
			10	25	Only conidiophores on both surfaces.
			12	100	Conidia abundant on both surfaces. July 14, no change.
			16	88	Conidia fairly abundant on both surfaces. July 21, 23, no change.
			25	50	Conidia few on both surfaces.
*4	12	1	21	50	Conidia few on both surfaces. July 23, no change.
			25	76	Conidia numerous on both surfaces.
5	1	2	1	10	No conidiophores on either surface. July 2, 7, 8, no change.
5	2	1	2	10	No conidiophores on either surface. July 7, no change.
			8	20	Conidiophores forming only on upper surface.
			10	30	Conidiophores above and conidia forming below.
			12	50	Conidia few on both surfaces.
			14	50	Conidia very few above, and fairly numerous below.
			16	50	Leaf dead, no change in spot.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
†5	4	3	July 7	20	No conidiophores above, very few conidia forming below.
			8	37	Conidiophores on upper surface, few conidia on lower.
			10	88	Conidia numerous above and very abundant below.
			12	75	Conidia few above and abundant below. July 14, no change.
			21	100	Conidia abundant on both surfaces. July 23 (leaf yellow), July 25, no change.
			28	63	Conidia few above and numerous below.
5	4	5	8	25	Conidiophores on both surfaces.
			10	37	Conidia few above and conidiophores below. July 12, no change.
			14	50	Conidia few on both surfaces.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	50	Conidia few on both surfaces.
			28	25	No conidia on either surface.
**5	6	1	7	17	Conidiophores none above and forming below.
			8	25	Conidiophores on both surfaces.
			10	75	Conidia few above and very abundant below. July 12, 14, 16, no change.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	57	Conidia very few above and numerous below.
			28	37	Conidia none above and few below.
5	8	4	14	10	No conidiophores on either surface.
			16	17	Conidiophores none above and forming below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	75	Conidia abundant above and few below.
*5	8	5	23	25	Conodiophores on both surfaces.
			25	75	Conidia numerous on both surfaces.
5	10	1	7	40	Conidia very few on either surface but more on lower. July 8, no change.
			10	75	Conidia few above and abundant below.
			12	100	Conidia abundant on both surfaces. July 14, 16, 21, no change.
5	10	2	7	35	Conidiophores forming above and conidia few below. July 8, no change.
			10	62	Conidiophores well developed above and conidia abundant below.
			12	70	Conidia very few above and abundant below.
			14	75	Conidia few above and abundant below. July 16, no change.
			21	100	Conidia abundant on both surfaces. July 23, no change.
5	10	3	14	10	No conidiophores on either surface.
			16	17	Conidiophores above and none below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	63	Conidia few above and numerous below.
*5	12	1	23	25	Conidiophores on both surfaces.
			25	70	Conidia very few above and abundant below.
			28	63	Conidia few above and numerous and matted below.
6	1	1	June 24	10	No conidiophores on either surface.
			25	20	Conidiophores very few above and abundant below. June 26, no change.
			27	31	Conidiophores above and conidia very few below. June 28, 30, July 1, 2 (leaf dying), no change.
			July 7	25	Conidiophores, but no conidia present.
7	6	1	7	10	No conidiophores on either surface.
			9	56	Conidia few above and fairly numerous below.
			10	62	Conidia none above and abundant below.
			12	100	Conidia abundant on both surfaces. July 14, 16, 21, 23, 25, no change.
7	6	3	10	15	Conidiophores forming on upper surface only.
			12	75	Conidia abundant above and few below.
			14	50	Conidia few on either surface. July 16, no change.
			21	100	Conidia abundant on both surfaces. July 23, 25, no change.
7	6	4	16	10	No conidiophores on either surface.
			21	100	Conidia abundant on both surfaces. July 23, 25, no change.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
*7	6	8	July 21	56	Conidia few above and fairly numerous below. July 23, no change.
			25	100	Conidia abundant on both surfaces.
*7	8	5	21	37	Conidia very few on both surfaces. July 23, no change.
			25	75	Conidia numerous on both surfaces.
			28	50	Conidia few on both surfaces. July 30, no change.
7	8	6	21	50	Conidia few on both surfaces.
			23	56	Conidia few above and fairly numerous below.
			25	63	Conidia numerous above and few below.
			28	50	Conidia few on both surfaces. July 30, Aug. 1, no change.
*7	8	7	July 23	15	Conidiophores none above and few below.
			25	75	Conidia few above and abundant below. July 28, 30, no change.
			Aug. 1	50	Conidia none above and numerous below.
7	8	12	July 25	25	Conidiophores on both surfaces.
			28	45	Conidia few forming on both surfaces.
			30	50	Conidia few on both surfaces. August 1, no change.
7	15	3	28	25	Conidiophores on both surfaces.
			30	63	Conidia few above and numerous below.
			Aug. 1	88	Conidia numerous above and abundant below.
†††8	8	5	July 9	10	No conidiophores on either surface.
			10	100	Conidia abundant on both surfaces.
*8	12	1	23	50	Conidia few on both surfaces.
			25	75	Conidia numerous on both surfaces. July 28, no change.
			30	88	Conidia numerous above and abundant below. August 1, no change.
			Aug. 5	75	Conidia numerous on both surfaces. Aug. 7, 9, 11, no change.
8	23	13	5	15	Conidiophores few above and none below. Aug. 7, no change.
			9	25	Conidiophores on both surfaces.
			11	57	Conidia numerous above and very few below.
			13	100	Conidia abundant on both surfaces.
†8	23	18	7	10	No conidiophores on either surface. Aug. 9, no change.
			11	60	Conidia abundant above and conidiophores few below.
			13	63	Conidia numerous above and few below. Aug. 15, 18, no change.
			22	100	Conidia abundant on both surfaces.
†8	24	1	July 28	75	Conidia numerous on both surfaces.
			30	62	Conidia fairly numerous on both surfaces. Aug. 1, no change.
			Aug. 5	44	Conidia few above and very few below.
			7	75	Conidia numerous and matted above and numerous below.
8	25	3	1	10	No conidiophores on either surface.
			5	44	Conidia very few above and few below.
			7	56	Conidia few above and fairly numerous below.
***8	29	1	1	10	No conidiophores on either surface.
			5	62	Conidia fairly numerous on both surfaces.
			7	75	Conidia numerous on both surfaces.
			9	50	Conidia few on both surfaces.
			11	56	Conidia few above and numerous below.
			13	100	Conidia abundant on both surfaces. Aug. 15, no change.
			18	75	Conidia few above and abundant below.
			22	88	Conidia numerous above and abundant below. Aug. 25, 28, no change.
			30	63	Conidia few above and numerous below. Sept. 1, no change except conidia matted above.
			Sept. 3	57	Conidia very few above and numerous below.
8	29	3	Aug. 5	25	Conidiophores on both surfaces.
			7	30	Conidiophores above and conidia forming below.
			9	50	Conidia very few above and fairly numerous below.
			11	100	Conidia abundant on both surfaces. Aug. 13, 15, 18, 22, 25, no change.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
***,†8	29	6	Aug. 5	20	Conidiophores very few on both surfaces. Aug. 7, no change.
			9	75	Conidia numerous on both surfaces. Aug. 11, no change.
			13	100	Conidia abundant on both surfaces. Aug. 15, no change.
			18	75	Conidia few above and abundant below.
			22	69	Conidia fairly numerous above and numerous below.
			25	100	Conidia abundant on both surfaces.
			28	87	Conidia fairly abundant on both surfaces.
			30	75	Conidia numerous on both surfaces.
			Sept. 1	69	Conidia fairly numerous above and numerous below.
			3	62	Conidia fairly numerous and matted above and fairly numerous below.
*** 8	29	8	Aug. 5	10	No conidiophores on either surface. Aug. 7, 9, no change.
			11	24	Conidia very few above and nothing below.
			13	88	Conidia abundant above and numerous below.
			15	94	Conidia fairly abundant above and abundant below.
			18	88	Conidia numerous above and abundant below.
			22	100	Conidia abundant on both surfaces.
			25	81	Conidia abundant above and fairly numerous and matted below.
8	29	9	5	35	Condiophores few above and conidia few below.
			7	50	Conidiophores numerous above and conidia numerous below.
			9	88	Conidia numerous above and abundant below.
			11	37	Conidia few above and none below.
			13	75	Conidia numerous and matted above and numerous below. Aug. 15, no change.
			18	88	Conidia numerous above and abundant below.
***.† 8	29	11	7	10	Conidiophores few on both surfaces.
			9	44	Conidia very few above and few below.
			13	75	Conidia numerous on both surfaces. Aug. 15, no change.
			18	88	Conidia numerous above and abundant below.
			22	63	Conidia very few and matted above and fairly abundant below.
			25	88	Conidia numerous and matted above and abundant below.
			28	75	Conidia few above and abundant below.
			30	88	Conidia numerous and matted above and abundant below.
			Sept. 1	82	Conidia numerous and matted above and fairly abundant below.
			3	69	Conidia few and matted above and fairly abundant below.
			6	50	Conidia very few and matted above and fairly numerous and matted below.
			8	43	Conidia none above and fairly numerous and matted below.
§8	33	1	1	10	No conidiophores on either surface.
			5	81	Conidia fairly numerous and matted above and abundant below. Aug. 7, no change.
			9	69	Conidia fairly numerous and matted above and numerous below.
			11	63	Conidia numerous above and few below.
			13	100	Conidia abundant on both surfaces.
			15	81	Conidia fairly numerous and matted above and abundant below. Aug. 18, no change.
			22	63	Conidia few and matted above and numerous below. Aug. 25, no change.
			28	50	Conidia none above and numerous below.
			30	56	Conidia none above and fairly abundant below.
†† 8	35	9	13	10	No conidiophores on either surface.
			15	15	Conidiophores few above and none below.
			18	37	Conidia very few on both surfaces.
			22	44	Conidia very few above and few below.
			25	62	Conidia fairly numerous on both surfaces.
			28	69	Conidia numerous above and fairly numerous below. Aug. 30, Sept. 1, no change..
			3	62	Conidia fairly numerous and matted above and fairly numerous below.
			6	50	Conidia few and matted on both surfaces.
			8	37	Conidia very few on both surfaces. Sept. 10, no change except matted on both surfaces.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
††,§8	§35	10	Aug. 13	10	No conidiophores on either surface.
			15	15	Conidiophores very few above and none below. August 18, no change.
			22	37	Conidia very few on both surfaces.
			25	56	Conidia fairly numerous above and few below.
			28	50	Conidia fairly numerous above and very few below.
			30	88	Conidia numerous and matted above and abundant below. September 1, 3, 6, no change.
			Sept. 8	50	Conidia few and matted on both surfaces.
			10	37	Conidia very few and matted on both surfaces.
††,§8	§37	1	Aug. 11	24	Conidia very few above and nothing below.
			13	100	Conidia abundant on both surfaces. August 15, no change except conidia matted above.
			18	94	Conidia fairly abundant and matted above and abundant below.
			22	88	Conidia numerous above and abundant below. August 25, no change.
			28	75	Conidia numerous on both surfaces.
			30	69	Conidia fairly numerous above and numerous below. September 1, 3, no change.
			Sept. 6	37	Conidia very few on both surfaces.
			8	25	Conidia none on either surface. September 10, 13, 15, no change.
††8	37	2	Aug. 11	10	No conidiophores on either surface.
			13	100	Conidia abundant above and abundant and matted below.
			15	94	Conidia abundant above and fairly abundant below.
			18	87	Conidia fairly abundant on both surfaces.
			22	100	Conidia abundant on both surfaces. August 25, no change except slightly matted on both surfaces.
			28	94	Conidia abundant and matted above and fairly abundant below.
			30	100	Conidia abundant and matted on both surfaces.
			Sept. 1	87	Conidia fairly abundant and matted on both surfaces. September 3, 6, no change.
			8	57	Conidia very few above and fairly numerous below. September 10, no change.
			13	37	Conidia none above and few below. September 15, no change, conidia still matted on both surfaces.
††8	37	3	Aug. 11	10	No conidiophores on either surface.
			13	18	Conidiophores very few on both surfaces.
			15	44	Conidia very few above and few below.
			18	50	Conidia very few above and fairly numerous below.
			22	62	Conidia fairly numerous and matted above and fairly numerous below.
			25	62	Conidia fairly numerous on both surfaces.
			28	75	Conidia numerous on both surfaces. August 30, no change, conidia matted on both surfaces.
			Sept. 1	62	Conidia fairly numerous and matted on both surfaces.
§8	37	6	Aug. 18	10	No conidiophores on either surface.
			22	50	Conidia few on both surfaces.
			25	62	Conidia fairly numerous on both surfaces.
			28	75	Conidia fairly abundant in center on both surfaces.
			30	70	Conidia numerous in center above and fairly abundant in center blow. September 1, no change.
			Sept. 3	69	Conidia fairly numerous above and fairly abundant in center below.
			6	87	Conidia fairly abundant on both surfaces.
			8	75	Conidia fairly numerous and matted above and fairly abundant below. September 10, no change.
			13	94	Conidia fairly abundant above and abundant below. September 15, no change.
			17	50	Conidia few on either surface.
8	38	1	Aug. 11	10	No conidiophores on either surface.
			13	69	Conidia numerous above and fairly numerous below.
			15	69	Conidia fairly numerous above and numerous and matted below.
			18	82	Conidia fairly abundant above and numerous and matted below.
			22	69	Conidia numerous and matted above and fairly numerous below.
8	39	6	Aug. 18	10	No conidiophores on either surface.
			22	63	Conidia numerous above and few below.
			25	82	Conidia fairly abundant above and numerous below.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
8	41	3	Aug. 22	31	Conidiophores above and conidia very few below.
			25	62	Conidia fairly numerous on both surfaces.
			28	82	Conidia numerous above and fairly abundant below.
			30	88	Conidia numerous above and abundant below. September 1, no change.
			Sept. 3	74	Conidia fairly numerous above and fairly abundant below.
			6	69	Conidia fairly numerous and matted above and numerous and matted below. September 8, 10, no change.
			13	57	Conidia very few and matted above and numerous below. September 15, 17, no change.
			19	50	Conidia none above and numerous below.
††, §8	44	1	Aug. 25	10	No conidiophores on either surface.
			28	24	Conidia very few above and nothing below.
			30	44	Conidia few above and very few below. September 1, 3, 6, no change.
			Sept. 8	50	Conidia few on both surfaces.
			10	56	Conidia fairly numerous above and few below.
			13	44	Conidia very few above and few below.
			15	69	Conidia fairly numerous and matted above and numerous below.
			17	44	Conidia very few and matted above and few below.
			19	31	Conidia none above and very few below.
†† 8	44	2	Aug. 25	15	Conidiophores forming above and nothing below.
			28	75	Conidia fairly abundant in center of both surfaces.
			30	90	Conidia abundant in center of both surfaces. September 1, 3, 6, no change.
			Sept. 8	69	Conidia fairly numerous above and numerous below.
			10	100	Conidia abundant on both surfaces. September 13, 15, no change.
			17	56	Conidia few and matted above and fairly numerous and matted below.
			19	50	Conidia very few and matted above and fairly numerous and matted below.
8	44	3	Aug. 25	10	No conidiophores on either surface.
			28	80	Conidia fairly abundant in center on both surfaces.
			30	90	Conidia fairly abundant in center above and abundant in center below. September 1, 3, no change.
			Sept. 6	87	Conidia fairly abundant and matted above and fairly abundant below. September 8, no change.
			10	94	Conidia fairly abundant above and abundant below. September 13, no change.
			15	88	Conidia numerous and matted above and abundant below.
			17	75	Conidia fairly numerous and matted above and fairly abundant below.
			19	63	Conidia few and matted above and numerous below.
††, §8	45	1	Aug. 22	37	Conidia very few on both surfaces.
			25	56	Conidia few above and fairly numerous and matted below.
			28	82	Conidia numerous and matted above and fairly abundant below.
			30	88	Conidia numerous above and abundant below. September 1, no change.
			Sept. 3	81	Conidia fairly numerous and matted above and abundant below. September 6, 8, no change except conidia matted below.
			10	75	Conidia fairly numerous and matted above and fairly abundant and matted below.
			13	69	Conidia few and matted above and fairly abundant and matted below.
			15	50	Conidia none above and numerous and matted below.
			17	31	Conidia none above and very few and matted below.
			19	25	Conidia none on either surface.
8	45	2	Aug. 25	10	No conidiophores on either surface.
			28	35	Conidia few above and conidiophores forming below.
			30	25	Conidia very few above and nothing below.
			Sept. 1	29	Conidia very few above and few conidiophores below. September 3, no change.
			6	44	Conidia few above and very few below.
			8	50	Conidia few and matted above and few below.
			10	44	Conidia few and matted above and very few below. September 13, 15, 17, no change.
			19	25	Conidia none on either surface.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
8	45	5	Aug. 25	10	No conidiophores on either surface.
			28	56	Conidia few above and fairly numerous below. August 30, September 1, 3, no change.
			Sept. 6	65	Conidia few and matted above and fairly abundant in center below.
			8	58	Conidia few and matted above and numerous in center below.
			10	69	Conidia few and matted above and fairly abundant below.
			13	63	Conidia few and matted above and numerous in center below. September 15, no change.
8	49	1	Aug. 28	37	Conidia very few on both surfaces.
			30	50	Conidia few on both surfaces. September 1, 3, no change.
			Sept. 6	69	Conidia numerous above and fairly numerous below.
			8	62	Conidia fairly numerous and matted above and fairly numerous below.
			10	75	Conidia numerous and slightly matted on both surfaces.
			13	56	Conidia fairly numerous and matted above and few below.
8	49	2	Aug. 28	10	No conidiophores on either surface.
			30	25	No conidiophores above and conidia very few below, September 1, 3, no change.
			Sept. 6	56	Conidia few above and fairly numerous below.
			8	50	Conidia very few above and fairly nnmerous below. September 10, 13, no change.
8	49	3	Aug. 28	10	No conidiophores on either surface.
			30	15	Conidiophores few above and very few below.
			Sept. 1	30	Conidiophores few above and conidia very few below. September 3, no change.
			6	62	Conidia fairly numerous on both surfaces.
			8	65	Conidia fairly numerous above and numerous in center below.
			10	75	Conidia numerous above and numerous and matted in center below.
			13	62	Conidia fairly numerous on both surfaces.
8	49	6	1	10	No conidiophores upon either surface. September 3, no change.
			6	44	Conidia very few above and few below. September 8, no change.
			10	50	Conidia few on both surfaces. September 13, no change.
8	49	7	1	10	No conidiophores on either surface. September 3, no change.
			6	24	Nothing above and conidia very few below.
			8	44	Conidia very few above and few below. September 10, 13, no change.
8	49	8	1	10	No conidiophores on either surface. September 3, 6, no change.
			8	20	Nothing above and conidia forming below.
			10	44	Conidia very few above and few below. September 13, no change.
8	49	9	3	10	No conidiophores on either surface. September 6, no change.
			8	20	Nothing above and very few conidia forming below.
			10	44	Conidia very few above and few below. September 13, no change.
8	49	10	3	10	No conidiophores on either surface. September 6, 8, no change.
			10	37	Conidia very few on both surfaces. September 13, no change.
8	51	1	3	10	No conidiophores on either surface. September 6, no change.
			8	24	Conidia very few above and nothing below. September 10, no change.
			13	37	Conidia very few upon both surfaces. September 15, 17, no change.
8	51	2	6	10	No conidiophores on either surface.
			8	37	Conidia very few on both surfaces.
			10	65	Conidia numerous in the center upon both surfaces. September 13, 15, no change.
			17	44	Conidia very few above and few below.
8	51	3	6	10	No conidiophores on either surface. September 8, no change.
			10	37	Conidia very few on both surfaces.
			13	44	Conidia few above and very few below. September 15, 17, no change.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
8	51	4	Sept. 6	10	No conidiophores on either surface. September 8, 10, no change.
			13	30	Conidiophores forming above and conidia very few below. September 15, 17, no change.
8	51	5	6	10	No conidiophores on either surface. September 8, 10, 13, 15, 17, no change.
8	51	6	6	10	No conidiophores on either surface. September 8, 10, 13, no change.
			15	15	Conidiophores none above and forming below.
			17	24	Nothing above and very few conidia below.
8	53	1	17	10	No conidiophores on either surface. September 19, no change.
8	53	2	17	10	No conidiophores on either surface. September 19, no change.
9	3	1	June 24	10	No conidiophores on either surface. June 25, 26, 27, 28, 30; July 1, 2, 7, no change.
**9	3	2	July 9	10	No conidiophores on either surface.
			10	25	Conidiophores abundant on both surfaces.
			12	87	Conidia fairly abundant on both surfaces. July 14, no change.
			16	100	Conidia abundant on both surfaces.
			21	37	Leaf dead, conidia very few on both surfaces. July 25, 28, no change.
9	5	1	21	50	Conidia few on both surfaces. July 23, 25, no change.
			28	37	Conidia none above and few below. July 30, no change.
*9	5	2	23	17	Conidiophores above and none below.
			25	75	Conidia numerous on both surfaces. July 28, 30, no change.
9	7	1	9	10	No conidiophores on either surface.
			10	37	Conidia few above and conidiophores abundant below.
			12	94	Conidia abundant above and fairly abundant below. July 14, 16, no change.
10	3	1	June 30	10	No conidiophores on either surface. July 1, 2, 7, 9, 10, 12, no change.
10	5	1	July 2	10	No conidiophores on either surface.
			7	20	Conidiophores few above and abundant below.
			9	37	Conidiophores above and conidia few below.
			10	75	Conidia numerous on both surfaces. July 12, 14, no change.
			16	100	Conidia abundant on both surfaces. July 21, 23, no change.
			25	75	Conidia few above and abundant below.
			28	50	Conidia none above and numerous below. Leaf dead. July 30, no change.
10	5	3	21	50	Conidia few on both surfaces. July 23, no change.
			25	56	Conidia fairly numerous above and few below.
			28	25	Conidia none on either surface. July 30, no change.
10	5	4	21	37	Conidiophores above and conidia few below.
			23	44	Conidia very few above and few below.
			25	37	Conidia very few on both surfaces.
			28	25	Conidia none on either surface. July 30, no change.
10	6	1	2	10	No conidiophores on either surface.
			7	31	Conidia very few above and conidiophores below.
			9	62	Conidia fairly numerous on both surfaces. July 10, 12, 14, no change.
			16	56	Conidia few above and fairly numerous below.
10	6	6	July 12	10	No conidiophores on either surface.
			14	15	Conidiophores none above and very few below.
			16	37	Conidiophores above and conidia few below.
			21	100	Conidia abundant on both surfaces. July 23, 25, 28, 30, no change.

TABLE IV.—*Data on life histories of a representative number of leaf spots studied on 10 plants in a medium-early sugar-beet field near Rocky Ford, Colo., during the season of 1913*—Continued

Plant No.	Leaf No.	Spot No.	Date.	Graph values.	Data on life histories.
			1913.		
**10	9	1	July 7	50	Conidia few on both surfaces. July 9, no change.
			10	75	Conidia few above and abundant below.
			12	94	Conidia fairly abundant above and abundant below. July 14, no change.
			16	69	Conidia very few above and abundant below.
			21	100	Conidia abundant on both surfaces. July 23, no change.
			25	75	Conidia numerous on both surfaces.
†10	9	2	7	37	Conidiophores above and conidia few below. July 9, no change.
			10	75	Conidia few above and abundant below.
			12	69	Conidia few above and fairly abundant below. July 14, no change.
			16	69	Conidia very few above and abundant below.
			21	100	Conidia abundant on both surfaces. July 23, 25, no change.
10	9	3	23	25	Conidiophores on both surfaces.
			25	75	Conidia numerous on both surfaces. July 28, no change.
*10	10	1	23	10	No conidiophores on either surface.
			25	75	Conidia numerous on both surfaces.
			28	75	Conidia abundant above and few and matted below. July 30, no change.
10	11	1	25	75	Conidia numerous on both surfaces.
			28	75	Conidia abundant above and few and matted below. July 30, no change.
10	11	3	25	50	Conidiophores above and conidia numerous below.
			28	75	Conidia numerous on both surfaces. July 30, no change.

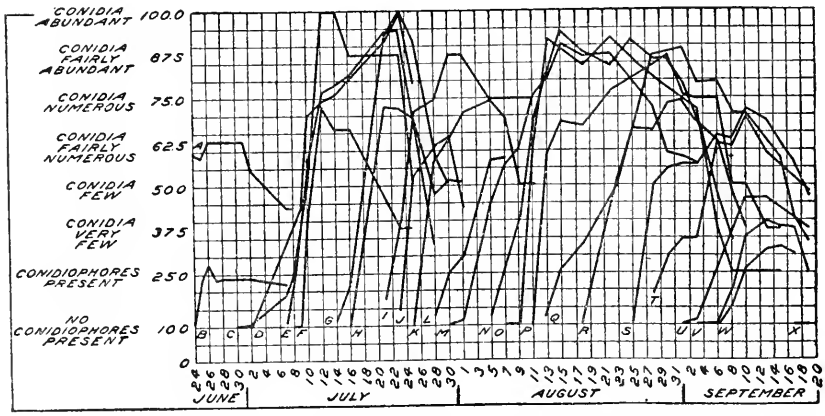


FIG. 6.—Curves of the leaf spot history series, showing the production of conidia on different dates from June 24 to September 19, 1913, at Rocky Ford, Colo.

After the values were all assigned, the spots which appeared on or about the same day were brought together in 24 groups and averaged (Table V and fig. 6). The temperature and humidity records used in the correlations with the leafspot histories were taken among the sugar-beet leaves near the surface of the ground.

TABLE V.—*Leafspot history series showing their arbitrary values on different dates and the number of spots entering into the average of each series at Rocky Ford, Colo., 1913*

Date.	Series and values.																							
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
June 24	59	11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	57.5	23	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26	62.5	27.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
27	62.5	23	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	62.5	23.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	62.5	23.5	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
July 1	62.5	23.5	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2	54	23.5	10	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	43.5	21.5	19	34	11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	43.5	.....	24	.....	20.5	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	.....	.....	.....	43	.....	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	.....	.....	50	70	56	41	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	.....	.....	100	74	77	73	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	.....	.....	100	76	79	66	11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
16	.....	.....	87.5	80.5	81.5	66	21	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
21	.....	.....	87.5	91	94	46	93.5	72.5	17.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	.....	.....	87.5	100	94.5	37.5	100	72	37.5	14	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	.....	.....	50	91.5	69	37.5	79	69	71	52	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	.....	.....	.....	58.5	33	.....	.....	47	75	61	58.5	12.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	.....	.....	.....	50	.....	.....	.....	51	87.5	64	63	25	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Aug. 1	.....	.....	.....	.....	.....	.....	.....	50.5	87.5	43.5	70.5	29.5	11.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	.....	.....	.....	.....	.....	.....	.....	.....	75	.....	75	57	45	12.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	.....	.....	.....	.....	.....	.....	.....	.....	75	.....	69.5	57.5	55.5	26	10	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	.....	.....	.....	.....	.....	.....	.....	.....	75	.....	50	.....	60.5	39	10	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	.....	.....	.....	.....	.....	.....	.....	.....	75	.....	50	.....	75.5	66.5	61.5	10	.....	.....	.....	.....	.....	.....	.....	.....
13	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	80.5	82	92	58.5	12	.....	.....	.....	.....	.....	.....	.....
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	90.5	94	89	68	25.5	.....	.....	.....	.....	.....	.....	.....
18	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	87	88	84.5	67	33	10.5	.....	.....	.....	.....	.....	.....
22	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	87.5	84	92.5	77	40.5	44.5	.....	.....	.....	.....	.....	.....
25	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	79.5	91.5	86	81	65.5	65.5	10.5	.....	.....	.....	.....	.....
28	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	72	86	81	84	65.5	87.5	50.5	19	.....	.....	.....	.....
30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	59	86	78	87.5	73.5	88	54.5	29.5	.....	.....	.....	.....
Sept. 1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	58	79.5	75	75	75	89.5	55.5	34.5	10	.....	.....	.....
3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	56	68.5	71.5	75	68.5	79.5	55.5	34.5	10	10	.....	.....
6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	47	37.5	75	62.5	79.5	64	62.5	26	10	10	.....
8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	34	25	50	48	70.5	63	61	36	19	15.5	.....
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25	50	37.5	70.5	71.5	70	46	35	26	.....
13	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25	37.5	.....	62.5	68.5	59	46	39.5	31	.....
15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25	37.5	.....	57.5	61.5	.....	.....	37.5	32	.....
17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	40.5	56	.....	.....	37.5	30	10
19	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	33.5	46.5	48	37.5	25	.....	10
Average number of spots	4	6	6	29	28	7	4	22	8	18	24	9	72	13	6	7	18	14	9	4	3	3	5	5

CONIDIAL PRODUCTION.—Under favorable conditions conidia are produced apparently much more readily by young than by old leaf-spots. For instance, as will be seen in Table IV, leaf spots (*)[1] 2 to 4 days old showed a marked increase in conidial production from July 23 to 25, while during the same period spots (**) 14 to 24 days old in most cases showed a decrease. However, spots (***) 2 to 3 weeks old on green leaves showed an increased production from August 18 to 25, the conditions being favorable, and some (†) even produced a second and third crop, although usually but one crop (††) is produced and this while the spots are comparatively young. It was also found that under favorable conditions a spot (†††) may produce abundant conidia on both surfaces in one day. Usually the maximum production is reached within 10 days after the spots appear (fig. 6), and sometimes under very favorable conditions the production may increase after this period (fig. 6, curves D and E, July 17 to 23), but the older spots do not always respond to favorable conditions in this way (fig. 6, curves C and F). In no case was a new growth of conidia observed on spots on yellow or dying leaves on green plants in the field. The fungus seemed to lose its vigor much sooner on such leaves than on green leaves which remained attached to the crown at harvest time. From the standpoint of control of the disease this is a very important point, from the fact that at harvest time the green leaves, on which the fungus is vigorous, are removed with the crowns and stored in the silo, while the yellow and dying leaves, on which the fungus may be too weak to overwinter, break off and remain on the ground.

During the greater part of August and September, when the precipitation was light (fig. 7), many of the conidia had a shrunken appearance and were massed together on the leafspot areas (Table IV,§). When placed in water, these conidia did not germinate; consequently this desiccation of the conidia may also be an important factor in connection with the vitality of the fungus on the host.

The position of the leaf on which the spot studied was located was also found to be an important factor in conidial production, an abundance of conidia being frequently observed on leaves protected from the sun, while at the same time few were observed on those exposed to the sun the greater part of the day. This difference in production is thought to be due mainly to the difference in humidity of the protected and the exposed locations.

A study of the comparative production of conidia on the upper and the lower surfaces of the spots was also made, the conidia on the spots included in series E, K, N, and S (fig. 6) being tabulated for this purpose.

Generally a more abundant conidial production was found on the lower than on the upper surface (fig. 8), and this was due apparently to

[1] The asterisks (*), daggers (†), and section marks (§) refer to particular leaf spots in Table IV.

the probably higher humidity of the former. Only during a very favorable period (fig. 7, July 19 to 21) or where the leaves were turned up or protected by other leaves was the conidial production on the upper surface equal to that on the lower surface (fig. 8, series E, July 21). At times, conidia were formed more abundantly on the upper surface than on the lower (fig. 6, series N, August 11, and series S, August 28). Because of the spongy parenchyma and the greater number of stomata on the lower surface, it might be supposed that conidiophores could be produced more readily on this than on the upper surface; but, as above indicated, humidity would seem to be the controlling factor in this connection.

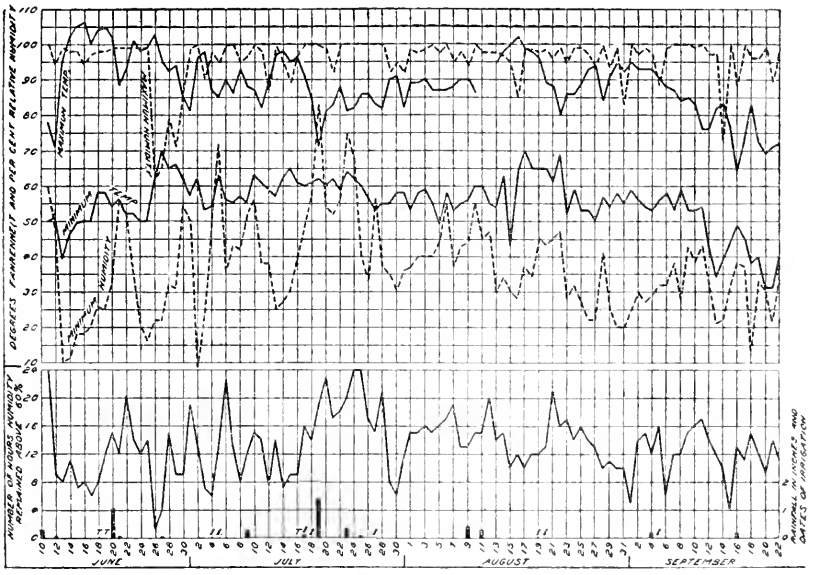


FIG. 7.—Curves of the maximum and minimum temperatures and humidities, the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among the plants, and rainfall and irrigation records, taken in a medium-early sugar-beet field from June 10 to September 22, 1913, at Rocky Ford, Colo.

A comparison of the conidial production as shown in Table IV and figure 6 and the climatic data shown in figure 7 indicates many definite relations. When the spots were first found, on June 20 and 24, conidia were fairly numerous (fig. 6, curve A) on all except six spots, which had evidently just developed on the latter date, as no conidia were present at this time and conidiophores only were produced the next two weeks. The following week there was but little increase, and during the next few days many of the conidia were disseminated. The small production of conidia was evidently due directly to the high temperature and the low humidity which prevailed during this period (fig. 7), as conidia were produced in great abundance from July 9 to 12 (fig. 6, curves C, D, E, F), when the temperature was lower and the humidity higher (fig. 7). Dur-

ing this time and the few days just preceding, the humidity remained above 60 for a longer time on an average and the minimum humidity did not become so excessively low nor the temperature so excessively high as during the time previous to July 4.

The next period of pronounced increase in conidial production was from July 19 to 23 (fig. 6, curves D, E, G), when the conditions were more favorable than during any period of similar length through the summer, the humidity ranging above 60 on an average of 19.4 hours each day and not falling below 52 (fig. 7), and the temperature ranging from 60° to 90° F.

Conidial production was again above the average (curves M, N, and O) from August 9 to 13, during which period the humidity remained above 60 from 13 to 20 hours each day and there was a small amount of rain which seemed to aid in maintaining the necessary humid conditions. Production was checked on August 16, on which date the temperature was 102° and the average humidity low, and was again inhibited after

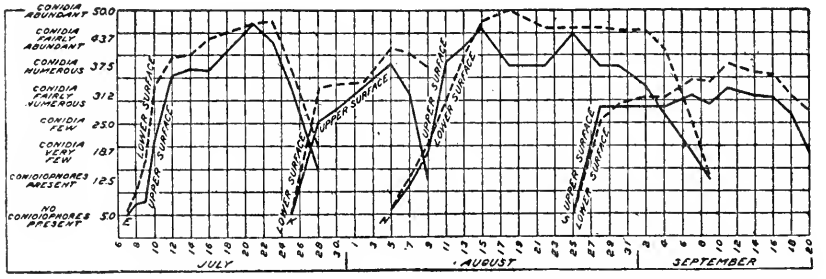


FIG. 8.—Curves of the comparative production of conidia on the upper and lower surfaces of the leaf spots, representing series E, K, N, and G of Table V and figure 6. Rocky Ford, Colo., 1913.

September 11, subsequent to which date the minimum temperatures ranged from about 30° to 45° and the maximum from about 65° to 83°, while the humidity remained above 60 for 12.4 hours per day, on an average.

The general conclusion from these tests is that conidial production is greatly influenced by temperature and relative humidity, or speaking specifically—

(1) A temperature of 100° F. or over is detrimental to conidial production, directly perhaps because it is inimical to the growth of the fungus and indirectly because humidity is ordinarily excessively low at such an extreme temperature.

(2) Conidial production is greatly checked at daily temperatures ranging below 50° as a minimum and 80° as a maximum.

(3) The most favorable temperature for conidial production is 80° to 90° in the daytime and not below 60° at night.

(4) The temperature being favorable, the largest conidial production occurred at the higher humidities. A good production occurred when

the humidity remained above 60 for not less than 15 to 18 hours, but very few were produced when the humidity remained above 60 for less than 10 to 12 hours daily.

With a view to determining the approximate number of conidia produced on a sugar-beet plant under a favorable temperature and humidity, one representing a heavy infection in August was selected. After the infected leaves were measured a representative portion of conidia were carefully washed off into sterilized water and counted. The count, which was made by means of a dilution method, showed 250,000,000 conidia on the plant at that time.

CONIDIAL DISSEMINATION.—That a period of low humidity, with its accompanying factors, is favorable to the dissemination of conidia was frequently observed (fig. 6, curve R). For instance, it was found that the amount of conidia diminished on September 1, 6, 14, and 15, when the humidity remained above 60 for 5, 6, 10, and 4 hours, respectively; while, on the other hand, there was no diminution in the amount present on September 3 to 5 and 8 to 10, during which periods the humidity remained above 60 for 12 to 16 hours.

Rainfall is also an important factor in the dissemination of conidia, as was noted in several instances. On July 19 (fig. 6, curve F) rain fell, and as a result many conidia were washed off, and the same was true in the case of rains on July 23 (curves C, D, E, G, H), August 9 (curve K), September 4 (curves N, O, R), and September 16 (curves Q, S, T, U, V, W). After rains on July 19, August 9, and September 4, however, there were more conidia present than before, but this was probably due to the fact that more were produced under the favorable humid conditions attending these rains than were washed off. It was also found that the conidia were disseminated more rapidly from the upper than from the lower surface of the spots (fig. 8). This was due probably to the greater exposure of the former to wind and rainfall.

RELATION OF INFECTION CYCLES TO CLIMATIC CONDITIONS

For the purpose of determining the relation of infection cycles to climatic conditions, a study was made of the increase and spread of disease in a field of sugar beets planted about May 1 [1] at Rocky Ford and one planted about two weeks later. Both fields had been in beets for two or three years, and as very few, if any, of the tops were removed after the harvest of 1912, infection appeared early in 1913 and was generally distributed.

Three plants in the early field (Table VII) and ten in the medium-early (Table VI) were selected, the leaves tagged and numbered consecutively, beginning with the outermost or oldest and continuing with the new ones as they appeared. The spots on each leaf were counted at

[1] Conidial production and dissemination were also studied in this field.

frequent intervals,[1] and the average actual increase of spots per plant computed (Table VIII and fig. 9). It was found that from 400 to 1,000 spots on a leaf, depending on its size, killed it within a few days.

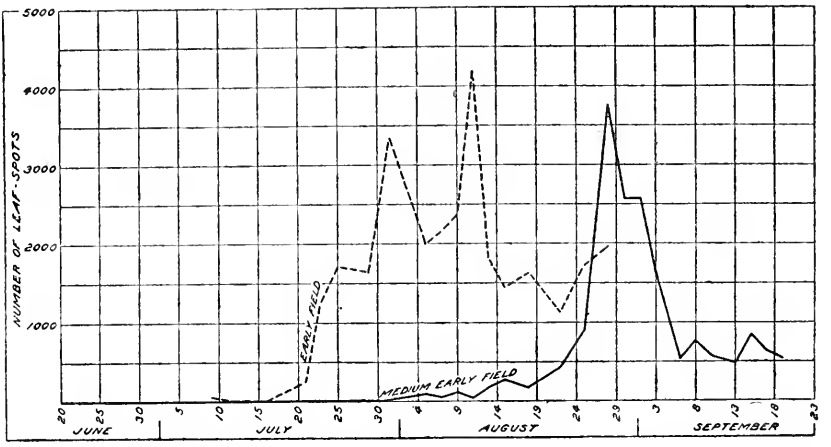


FIG. 9.—Curves of the 2-day average increases in the number of leaf spots per plant in a medium-early and an early sugar-beet field, from June 18 to September 19, 1913, at Rocky Ford, Colo.

TABLE VI.—*Average infection cycle of Cercospora beticola in a medium-early sugar-beet field with poorly developed foliage and with a consequent low humidity early in the season at Rocky Ford, Colo., in 1913*

Date.	Total number of leaves marked.	Total number of leaves infected.	Total number of leaves dead.	Number of leaves killed by *Cercospora beticola*.	Number of infected green and dying leaves.	Number of functional leaves— Infected.	Number of functional leaves— Uninfected.	Total number of functional leaves.	Total number of leaf spots per plant.	Average number of leaf spots per leaf.
July 2	14.2	3.2	3.3	.....	3	2.9	8	10.9	5	1.6
7	16.8	4.7	3.8	.....	4.4	4.1	8.9	13	10.4	2.3
8	17.4	5.4	4.3	.....	4.7	4.4	8.7	13.1	11.4	2.4
10	18.5	5.5	4.6	.....	4.5	4.3	9.6	13.9	11.2	2.4
12	19.7	5.5	5.1	.....	4.3	4	10.6	14.6	10.8	2.5
14	21.3	5.8	5.5	.....	4.3	4	11.8	15.8	10.8	2.5
16	22.6	6	6.1	.....	4.1	3.8	12.7	16.5	10.4	2.5
21	26.3	6.6	7.1	.....	4.4	3.5	15.7	19.2	13.3	3
23	27.5	7.8	8	.....	4.7	4.1	15.4	19.5	17.2	3.6
25	29	8.1	8.9	.....	4.4	3.5	16.6	20.1	17.5	3.9
28	30.5	10.8	9.3	.....	6.3	5.8	15.4	21.2	20.5	3.2
30	33	17.6	11	.....	10.7	10.3	11.7	22	42	3.9
Aug. 1	34	27	12	.....	20	19	3	22	93.5	4.7
5	35.5	27	12.5	.....	19	18.5	4.5	23	292	15.3
7	36.5	27	13.5	.....	18.5	17.5	5.5	23	355	19.1
9	37.5	29	13.5	.....	19.5	19.5	4.5	24	402	20.6
11	38.5	30	15.5	.....	20.5	19	4	23	455.5	22.2
13	40	31	15.5	.....	20	20	4.5	24.5	593	29.6
15	41.5	31.5	15.5	.....	20.5	20.5	5.5	26	875	42.6
18	43	33.5	15.5	.....	22.5	22.5	5	27.5	1,155.5	51.3
22	45	35.5	15.5	.....	24.5	24.5	5	29.5	2,045.5	85.9
25	46	38	15.5	.....	27	27	3.5	30.5	3,420	126.6
28	48	41	17	1.5	29.5	28	3	31	9,081.5	307.8
30	48.5	41	18	2	28.5	27.5	3	30.5	10,856	380.9
Sept. 3	50	41.5	23.5	6	25.5	22.5	4	26.5	12,371.5	485.1
6	50.5	42.5	25.5	8	23.5	21.5	4	25	11,493	489
8	51.5	42.5	26.5	9	21.5	20.5	5	25	10,928.5	508.3
10	52.5	44.5	28.5	11	22.5	20.5	4.5	24	10,782	479.2
13	53	44.5	30	12.5	20.5	19	4	23	10,003.5	487.9
15	53.5	44.5	32.5	15	19	16.5	4.5	21	9,585.5	504.5
17	54	45.5	34.5	17	17.5	15.5	4	19.5	8,504	485.9
19	55	45.5	35	17.5	15.5	15	5	20	7,597.5	490.1

[1] For convenience and uniformity, 2-day averages were used in making the comparisons, the counts being made usually at 2-day intervals.

TABLE VII.—*Average infection cycle of Cercospora beticola in an early sugar-beet field in which there was a heavy production of foliage and a consequent high humidity early in the season at Rocky Ford, Colo., in 1913*

Date.	Total number of leaves marked.	Total number of leaves infected.	Total number of leaves dead.	Number of leaves killed by *Cercospora beticola*.	Number of infected green and dying leaves.	Number of functional leaves—		Total number functional leaves.	Total number of leaf spots per plant.	Average number of leaf-spots per leaf.
						Infected.	Uninfected.			
July 7	23	13.5	4	.....	12	12	7	19	529	44
9	24	13.5	4.5	.....	12	11.5	8	19.5	596.5	49.7
12	26	14	5.5	.....	12	11	9	20.5	615	51.2
14	27.5	14.5	5.5	.....	11.5	11.5	10.5	22	554.5	48.2
16	29.5	16	7	.....	13	11.5	10.5	22.5	574.5	44.2
21	34.5	23	8.5	.....	18.5	17	9	26	1,113.5	60.2
23	37	29	10.5	.....	23	21	5.5	26.5	2,216.5	96.3
25	38.5	30	11	.....	22	21.5	6	27.5	3,776.5	171.6
29	41.5	31	11.5	.....	22.5	22	8	30	7,045.5	313.1
Aug. 1	44	37	15	3	28	24.5	4.5	29	11,966.5	427.3
5	46.5	39	16	4	26.5	25.5	5	30.5	12,638	476.9
7	48.5	40	18	6	26.5	24.5	6	30.5	13,905	524.7
9	50	42	20	8	26.5	24.5	5.5	30	14,228.5	536.9
11	51.5	42	21.5	9.5	24.5	23.5	7	30	16,386	668.8
13	53.5	42.5	24.5	12.5	23.5	20.5	8.5	29	16,693	710.3
15	55.5	45	29.5	17.5	23	18	8	26	14,993	651.8

TABLE VIII.—*Actual and 2-day average increase in the number of leaf spots per plant in a medium-early and an early sugar-beet field from June 18 to September 19, at Rocky Ford, Colo., in 1913*

Date.	Increase in medium-early field.		Increase in early field.		Date.	Increase in medium-early field.		Increase in early field.	
	Actual.	2-day average.	Actual.	2-day average.		Actual.	2-day average.	Actual.	2-day average.
June 20	0.3	0.3	.....	.....	Aug. 5	203	102	3,996	1,998
22	.5	.5	.....	.....	7	65	65	2,167	2,167
24	0	0	.....	.....	9	127	127	2,373	2,373
26	.3	.3	.....	.....	11	53	53	4,208	4,208
28	.1	.1	.....	.....	13	190	190	1,831	1,831
30	.4	.4	.....	.....	15	282	282	1,450	1,450
July 2	2.3	2.3	.....	.....	18	280	186	2,450	1,633
7	4.9	1.9	.....	.....	22	895	447	2,286	1,143
8	1.4	2.8	.....	.....	25	1,379	919	2,582	1,722
9	.....	.....	70	70	28	5,664	3,776	2,944	1,962
10	.9	.9	.....	.....	30	2,572	2,572	.....	.....
12	.2	.2	18.5	12.2	Sept. 1	2,582	2,582	.....	.....
14	.7	.7	24.5	24.5	3	1,634	1,634	.....	.....
16	.7	.7	20.5	20.5	6	799	532	.....	.....
21	3.3	1.3	672	269	8	771	771	.....	.....
23	3.3	3.3	1,272	1,272	10	578	578	.....	.....
25	1.9	1.9	1,723	1,723	13	732	488	.....	.....
28	4.9	3.2	.....	.....	15	857	857	.....	.....
29	.....	.....	3,282	1,641	17	635	635	.....	.....
30	5.1	5.1	.....	.....	19	543	543	.....	.....
Aug. 1	44	44	4,978	3,318					

The period of incubation of the fungus being from 11 to 13 days, as shown by artificial infection experiments, a corresponding increase in the number of spots on the leaves would not necessarily follow immediately after a period during which conditions favorable for infection prevailed.

Notwithstanding the early appearance of the spots in the medium-early field—on June 20—and a consequent expectation of an epidemic of the disease, the increase in infection was very light (Table VIII)

during the latter part of June and early part of July. This was doubtless due to the fact that during this period the stomata were closed against the fungus the greater part of the day on account of the excessively high temperature, which was generally above 100° F., and the excessively low humidity, which at one time fell to 10 and which was only above 60 from 6 to 15 hours a day, and also to the fact that a temperature as high as 95° inhibits the growth of the fungus and kills it after a few days.

After July 5 the temperature was lower and the humidity higher than during the period above mentioned. As a result, numerous conidia were produced from July 9 to 12, and at the end of the period of incubation—July 21 to 25—there was a slight increase in the number of spots (Table VIII). Rains between July 19 and 25 and the resulting high humidity caused a rather marked increase in the number of spots in late July and early August, these spots appearing on many leaves hitherto uninfected (Table VI). Prior to this period the number of leaves showing spots were comparatively few, but after July 30 the majority showed spots, and the proportion of infected to uninfected leaves gradually increased until August 28, after which it decreased. During the period from July 30 to August 28 the humidity was comparatively high, remaining above 60 on an average of 14.6 hours on all except three days, on which it remained above 60 for 10 hours; and the maximum and minimum temperatures generally were not above 90 nor below 55, respectively. The increased proportion of infected to uninfected leaves during this period, however, was not necessarily due to increasingly favorable climatic conditions but to the cumulative effect of the organism, the amount of viable conidia and consequent new infections increasing as the number of spots increased, as shown by the enormous increase of 3,776 spots per plant on August 27 and 28. After September 3 the increase in infection was considerably less (fig. 9). This was due apparently to the fall in temperature, the maximum being rarely above 76° F and the minimum seldom above 50° after September 8, while the humidity was comparatively favorable.

The increase in infection through the season was considerably higher in the early than in the medium-early sugar-beet field, as shown by the total amount of the disease (Tables VI and VII) and the actual increase (Table VIII and fig. 9). This was due to the fact that the foliage was heavier in the early than in the medium-early field (Tables VI and VII, functional leaves), and consequently the humidity was higher and the infection greater in the former than in the latter (Table VIII and fig. 9).

The maximum increase in spots was reached on August 11 in the early field and on August 28 in the medium-early sugar-beet field. The period of greatest increase in the disease is not its period of greatest destructiveness, however, as the plant is not immediately affected by the disease, some time being required for the leaves to be killed.

Prior to August 1, only isolated records of humidity were made in the early field,[1] but after this date continuous records of both humidity and temperatures in both fields were available for comparison (fig. 10).[2] The temperatures prevailing in the two sugar-beet fields were quite comparable, but the humidity was generally different. For instance, from August 2 to 23 the humidity remained above 60 for a longer time, and the maximum humidity was, as a rule, higher in the early than in the medium-early field; from August 23 to September 1 the maximum humidity was lower in the early than in the medium-early field; after the latter date strikingly lower, the difference ranging from 5 to 15 units; after September 5 the humidity remained above 60 for a shorter time in the former than in the latter field; but from September 6 to 21 the range of humidity in the two fields was much closer than during the periods previously mentioned.

The difference in the humidity of the two fields seemed to be due to the difference in the amount of foliage present. Early in the experiment the foliage was heavier in the early than in the medium-early field, but owing to an extremely severe infection, which developed between July 29 and August 13 (fig. 9), the relative proportion of foliage in the two fields was reversed after that period. As a result of this reversal, less moisture was retained and the humidity was lower in the early than in the medium-early field during September, and consequently at that time the relative increase in infection was less in the former than in the latter. Speaking more specifically, early in the experiment there was an average of 29 functional leaves per plant in the early field and 22 in the medium-early; on August 15 there was an average of 26 leaves per plant in both fields, while later on there were fewer per plant in the early than in the medium-early field. On the other hand, on August 13 there was an average of 23.5 infected leaves per plant, with an average of 710 spots per leaf in the early field, and on September 8 there was an average of 21.5 infected leaves per plant, with an average of 508.3 spots per leaf, in the medium-early field.

A comparison of the death rate of the leaves in the two fields before and after the disease appeared shows its destructiveness. For instance, in the early and medium-early fields, from July 7 to 29 and from July 2 to August 25, when no leaves were killed by the fungus, the death rate from normal causes was approximately one leaf per plant in three and four days, respectively; while from July 29 to August 15 and August 25 to September 19, when the disease was most severe in the two fields, the death rate averaged one leaf per plant in nine-tenths of a day and one and three-tenths days, respectively.

[1] These and the later continuous records indicate that prior to August 1 the humidity was generally higher in the early than in the medium-early field.

[2] The temperature records taken at the Weather Bureau station were included in the comparisons and were found to agree closely with those obtained in the two fields.

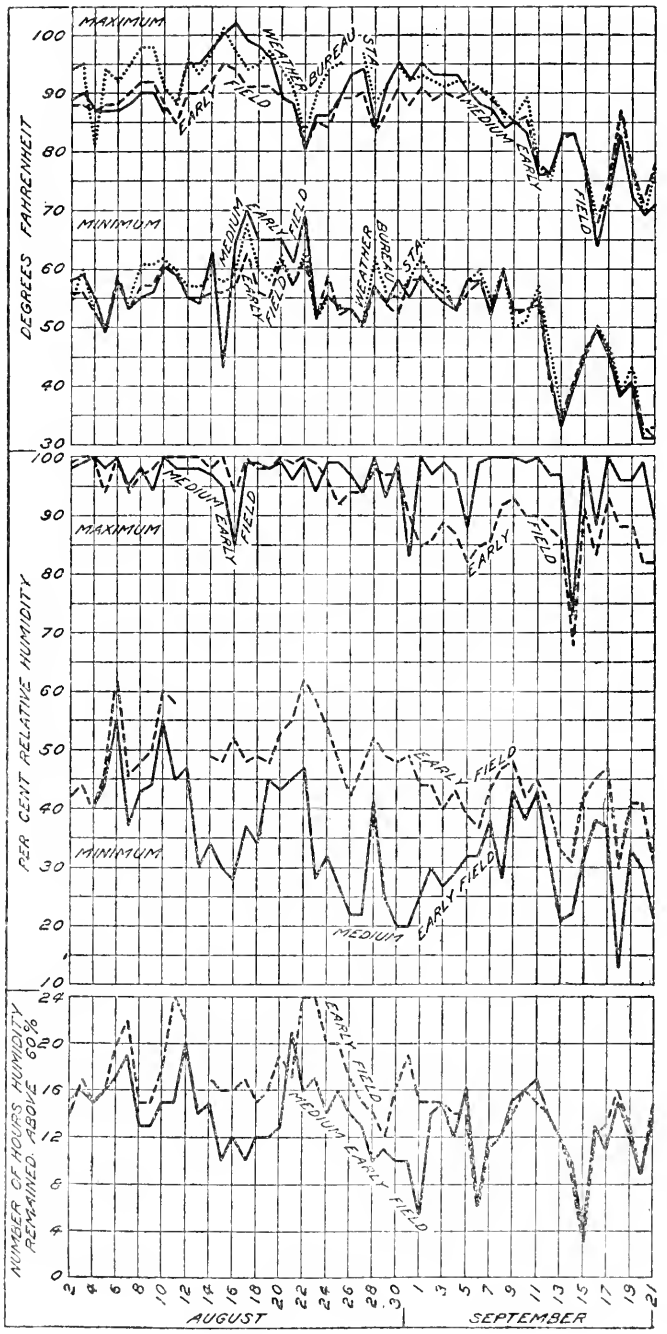


FIG. 10.—Curves of the maximum and minimum temperatures and relative humidities and the number of hours that the humidity remained above 60 from noon of the preceding to noon of the given day among the sugar-beet plants of a medium-early and an early field. For comparison, the maximum and minimum temperature records from the Weather Bureau station are included. August 2 to September 21, 1913, at Rocky Ford, Colo.

SUMMARY

(1) The life of the fungus *Cercospora beticola* overwintering in sugar-beet-top material varies with different environment. When exposed to outdoor conditions, the conidia die in from one to four months; but when kept dry live as long as eight months. The sclerotia-like bodies, which are more or less embedded in the tissues of the host, are more resistant than the conidia, living through the winter when slightly protected, as, for instance, in the interior of a pile of hayed sugar-beet tops or buried in the ground from 1 to 5 inches, and become a source of infection for the succeeding crop. Notwithstanding the difference in temperature and soil-moisture conditions, similar results from the overwintering experiments were obtained at Rocky Ford, Colo., and Madison, Wis.

(2) Climatic conditions and the development of the leafspot can be correlated only when all records are taken at the same relative positions, as shown by comparisons of the Weather Bureau records and the records taken among the plants and 5 feet above the field.

(3) The maximum temperature is much higher near the ground than 5 feet above early in the season, but the difference diminishes as the season advances.

(4) Throughout the season the maximum relative humidity was higher among the leaves than 5 feet above the field. Early in the season, while the plants were small, the humidity remained above 60 longer each day 5 feet above the field than among the plants near the ground; but after the plants attained a good size this condition was reversed. Because of this difference, only records collected among the leaves should be considered in correlating climatic conditions and conidial production and infection.

(5) The effect of rainfall and irrigation on the increase of relative humidity and its duration is apparently much the same.

(6) Thermal tests with artificial cultures showed (a) that exposure to constant temperatures of 35° and 36° C. is fatal to the growth of the fungus; (b) that growth occurred when cultures after exposure for 3 days to either of these temperatures were changed to 30.8°, and also when they were held at either for 8 hours and then at 20° for 16 hours; and (c) that a temperature of 40.5° was fatal in all combinations tested.

(7) Temperature and relative humidity influence the production of conidia and infection in much the same way. A temperature of 80° or 90° F., with a night minimum preferably not below 60°, is most favorable to conidial production, while it is checked by a temperature of 100° or higher and greatly checked by a range from below 50° to 80°. A maximum humidity ranging above 60 for not less than 15 to 18 hours each day induces a good growth of the fungus.

(8) Because of the higher humidity on the lower than on the upper surface of the leaf, the conidia are generally more abundant on the lower surface of the spots, but because of the action of rain and wind they disappear more rapidly from the upper surface.

LITERATURE CITED

FRÖDIN, John.
1913. Beobachtungen über den Einfluss der Pflanzendecke auf die Bodentemperatur. *In* Lunds Univ. Årsskr., n. f. afd. 2, bd. 8, no. 9, 16 p., 1 fig., 4 pl. Literatur, p. 15.

MASSEE, George.
1906. Plant diseases.—IV. Diseases of beet and mangold. *In* Roy. Gard. Kew, Bul. Misc. Inform., 1906, no. 3, p. 49–60, 5 fig.

PAMMEL, L. H.
1891. Fungus diseases of sugar beet. *In* Iowa Agr. Exp. Sta. Bul. 15, p. 234–254, 7 pl.

POOL, Venus W., and McKAY, M. B.
1915. Phoma betae on the leaves of the sugar beet. *In* Jour. Agr. Research, v. 4, no. 2, p. 169–178, pl. 27.

——— ———

1916. Relation of stomatal movement to infection by Cercospora beticola. *In* Jour. Agr. Research, v. 5, no. 22, p. 1011–1038, 6 fig., pl. 80–81.

SHAW, H. B.
1914. An improved cog psychrometer. *In* Plant World, v. 7, no. 6, p. 183–185, 2 fig.

STEWART, F. C.
1913. The persistence of the potato late-blight fungus in the soil. N. Y. Agr. Exp. Sta. Bul. 367, p. 357–361.

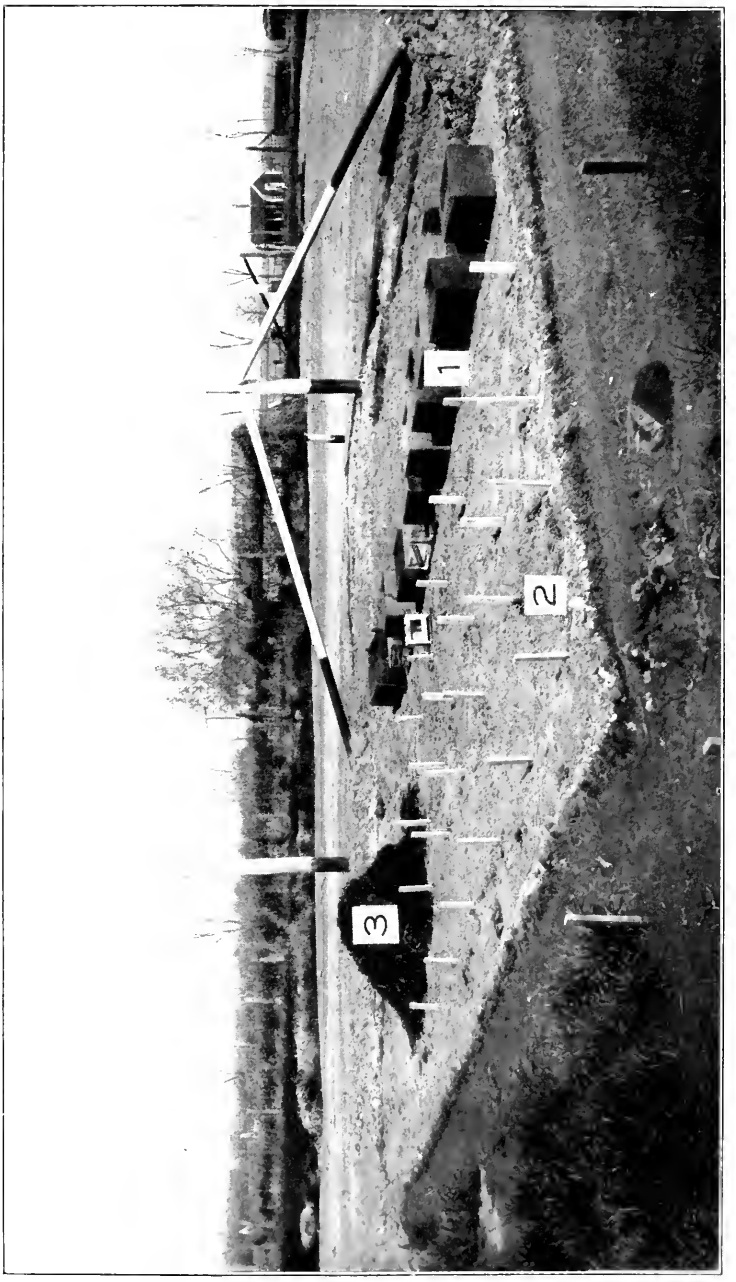
THÜMEN, Felix von.
1886. Die Bekämpfung der Pilzkrankheiten unserer Culturgewächse. 157 p. Wien.

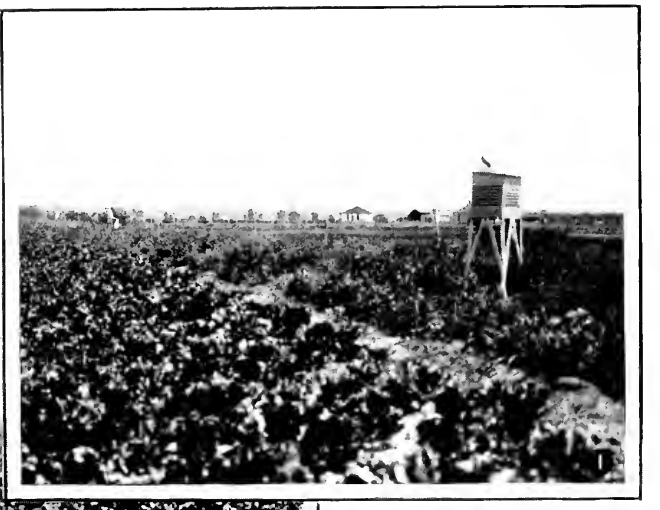
TREBOUX, O.
1914. Überwinterung vermittels Mycels bei einigen parasitischen Pilzen. *In* Mycol. Centbl., Bd. 5, Heft 3, p. 120–126.

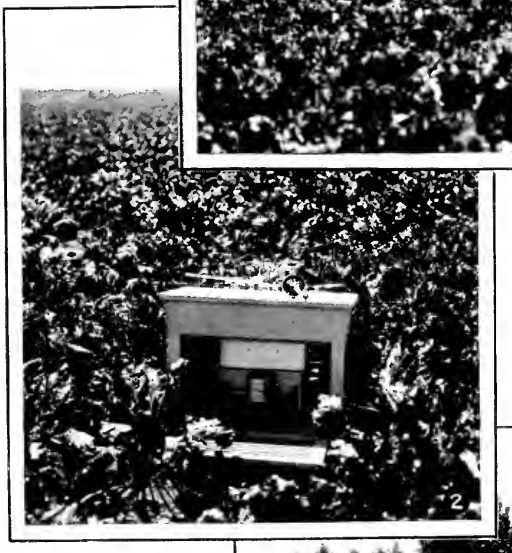
PLATE III

Cercospora beticola: Overwintering tests on the experimental field at Rocky Ford, Colo., during 1912–13:

Sugar-beet leaves infected with *Cercospora beticola* (*1*) stored in soil in boxes, (*2*) buried in the ground at different depths from 1 to 8 inches, and (*3*) left exposed above the ground in a pile of hayed sugar-beet tops.


1
2
3





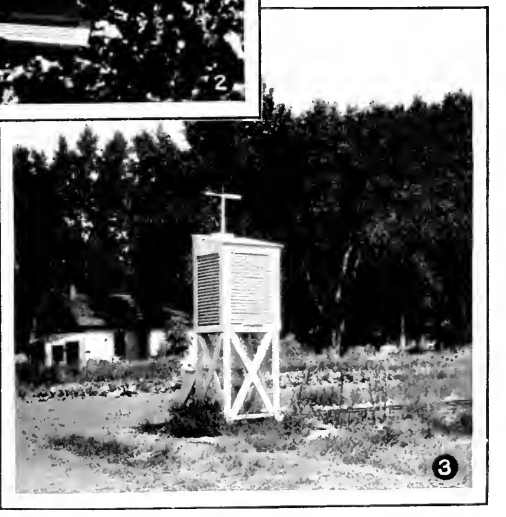


PLATE IV

Field stations for the collection of weather data at Rocky Ford, Colo., in 1913:

Fig. 1.—Weather shelter, anemometer, and rain gauge at edge of sugar-beet field.

Fig. 2.—Weather shelter among beet plants, showing hygrothermograph and cog psychrometer.

Fig. 3.—Weather shelter of the local Weather Bureau station about 3 miles from sugar-beet field.

27469°—16——5

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
20 CENTS PER COPY
SUBSCRIPTION PRICE, 3.00 PER YEAR

Vol. VI APRIL 10, 1916 No. 2

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., APRIL 10, 1916 NO. 2

SOLUBLE NONPROTEIN NITROGEN OF SOIL

By R. S. POTTER, *Assistant Chief in Soil Chemistry*, and R. S. SNYDER, *Assistant in Soil Chemistry, Iowa State College Experiment Station*

INTRODUCTION

Dilute alkali dissolves a larger proportion of the organic material of soil than any of the other relatively mild reagents. A still larger percentage is extracted from soil previously treated with 1 per cent of hydrochloric acid (HCl), and this latter reagent dissolves but little of the organic material. The term "humates" is fast disappearing from current scientific literature, yet one often reads that the reason the preliminary washing with acid renders the organic matter more soluble in the alkali is that the calcium of the calcium humates is dissolved out, making the free humic acids soluble in the alkali. To say that the proteins are rendered more soluble by the removal of the calcium and the heavy metals would explain the solubility just as well and would be more correct scientifically.

As pointed out by Lipman (4, p. 251), much of the organic nitrogen of the soil must be protein in nature. The chief sources of the nitrogen are crop residues, manures, and bacterial cells, and in these much of the nitrogen is in the form of protein. Investigations carried out in this laboratory (5) have shown that soils contain a large quantity of the so-called humin compounds. These have a great tendency to be adsorbed by such compounds as magnesium oxid and calcium hydroxid, and therefore removal of calcium from the soil by acid would tend to make these more soluble.

Upon the acidification of the alkali extract a precipitate is obtained which has been called humic acid. This term also is no longer taken seriously. It would seem that the rational explanation of this precipitate would be simply that it was made up of proteins thrown down, as salts of the precipitant, as salts of organic acids, such as nucleic acid (7), or resinous acids (6), both of the latter substances having been isolated from the acid precipitate. It would also contain, no doubt, some free organic acids.

In analyses of the solution obtained by the prolonged boiling of soils with strong acids and of the hydrolyzed humic acids by the Van Slyke method (8), it was found that the results for the humic acids did not differ markedly from the results for the organic matter of the soils as a whole from which they were derived. Since that time it has occurred to

the writers that this would hold for the material precipitated by acid from the alkali extract, but perhaps this would not be true of the organic nitrogen compounds remaining in solution. It has been pointed out by Shorey (7) that many organic compounds have been isolated from the alkali extract of soil, which, though relatively quite soluble in water, can not be detected in or isolated from the water extract of soils. Therefore it has seemed that information might be obtained relative to the degree of decomposition of the organic matter in the soil by determining the proportion of nitrogenous compounds left in solution after the precipitation of the proteins by a suitable reagent was completed. It was with these problems in mind that the preliminary investigation was carried out.

EXPERIMENTAL METHOD

The general procedure followed was to determine the nitrogen in the alkali extract of soil with and without added material and the determination of nitrogen in the filtrate from the precipitate of the proteins in the alkali extract of soil with and without added material. The recent critical examination of a few protein precipitants by Greenwald (3) led us to use trichloracetic acid as our precipitant.

The detailed procedure was as follows: Soil, ground to pass a sieve of 100 meshes to the inch, was extracted with 1 per cent of hydrochloric acid until no calcium was found in the wash water. After air drying, 100 gm. were placed in an 800 c. c. bottle and 500 c. c. of a 1.5 per cent solution of sodium hydroxid added. After shaking the mixture for 2 hours it was centrifuged for 5 minutes in a bowl centrifuge having a speed of 18,000 revolutions per minute. Two 25 c. c. portions of the clear but deeply colored extract were analyzed for total nitrogen by the Gunning-Arnold method. Two 25 c. c. portions were neutralized with sulphuric acid, sufficient trichloracetic acid in solution added to give a 2.5 per cent solution of the acid and a total volume of exactly 30 c. c. After centrifuging, 10 c. c. portions were taken from each tube and analyzed for nitrogen by the Bock and Benedict (1) modification of the Folin and Denis method (2). This was called the soluble nonprotein nitrogen.

The same procedure was used where material was added to the soil. In the case of guanin, hypoxanthin, and glucosamin,[1] weighed portions of the compounds were added to the soil, which was then shaken with the alkali. The hydrolyzed casein was prepared as directed by Greenwald (3), which consisted, in brief, of boiling the casein for 40 hours with hydrochloric acid, the removal of the acid under diminished pressure, neutralization with sodium hydroxid (NaOH), and filtration. After mixing the filtrate with animal charcoal it was again filtered and final filtration carried out after crystallization of the tyrosin. For all the remaining materials solutions were prepared, sometimes with the aid of a little *N*/10 acid or *N*/10 alkali. Suitable amounts of the solutions were

[1] We wish to express our thanks to Prof. P. A. Kober for the samples of the hypoxanthin and guanin, and to Dr. A. W. Dox for the sample of glucosamin.

added to the soils and then sufficient alkali added to make 500 c. c. of a 1.5 per cent solution. In all cases, with the above-noted exceptions, the purest commercially available compounds were used, but analyses for nitrogen were run on the solid material when it was used, and when solutions were employed aliquots were analyzed. These determinations were also made by the micro method. It should be mentioned here that 6 minutes was found to be quite an inadequate digestion period for some of the compounds. It is believed that in some cases when apparently more than 100 per cent of the added substance was extracted from soil, faulty analysis of the substance was the cause. Insufficiency of material precluded repeating tests with many of the materials.

The soil used for all these tests was a silt loam containing 0.30 per cent of nitrogen. Samples A and B, as shown in Table I, differ only in that they were not taken from the field at the same time.

TABLE I.—*Analyses of 5-gm. portions of soil for alkali-soluble and soluble nonprotein nitrogen*

Soil sample.	Substance added.	Nitrogen added.	Nitrogen in the alkali extract.	Nitrogen of the added substance recovered in the alkali extract.		Soluble nonprotein nitrogen.	Soluble nonprotein nitrogen recovered.	
		Mgm.	*Mgm.*	*Mgm.*	*Per ct.*	*Mgm.*	*Mgm.*	*Per ct.*
A	Nothing	.....	5.93	.....	.....	1.29	.....	.....
	Hydrolyzed casein	2.09	7.99	2.06	98.4	3.33	2.04	97.5
	Amino benzoic acid	2.14	8.08	2.15	100.4	3.41	2.12	99.0
	Glutamic acid	2.27	8.26	2.33	102.6	3.48	2.19	96.5
	Hippuric acid	2.42	8.40	2.47	102.0	3.73	2.44	100.8
	Glutamic acid imid	2.36	8.36	2.43	103.0	3.59	2.30	97.5
	Succinimid	2.53	8.45	2.52	99.5	3.86	2.57	101.5
	Guanidin sulphate	1.97	6.725	1.975	40.4	1.84	0.55	28.0
	Urea	2.48	8.41	2.48	100.0	3.66	2.37	95.6
	Uric acid	2.42	8.34	2.41	99.6	3.78	2.49	103.0
	Caffein	2.32	6.93	1.00	43.1	2.21	.92	39.7
	Theobromin	1.96	7.90	1.97	100.5	3.27	1.98	101.0
	Guanin	2.49	8.45	2.52	101.2	2.325	1.035	41.6
	Hypoxanthin	2.52	8.34	2.42	96.0	3.42	2.13	84.6
	Skatol	1.26	7.19	1.26	100.0	1.80	.51	41.2
	Nucleic acid	2.00	7.92	1.99	99.5	1.67	.38	19.0
	Cadaverin	2.00	7.92	1.99	99.5	2.45	1.16	58.0
	Amygdalin	2.48	8.41	2.48	100.0	3.42	2.13	85.7
	Peptone (Witte)	2.98	8.76	2.83	94.9	2.12	.83	33.5
	Casein	2.42	7.42	1.49	61.6	1.29	0	0
	Edestin	2.43	6.58	.65	26.8	1.29	0	0
	Egg albumin	2.01	7.56	1.63	81.3	1.29	0	0
	Glucosamin	1.98	8.08	2.15	108.5	3.36	2.07	104.5
B	Nothing	.....	6.51	.....	.....	1.25	.....	.....
	Asparagin	2.11	8.62	2.11	100.0	3.24	1.99	94.2
	Acetanilid	2.14	8.66	2.15	100.5	3.24	1.99	93.0
	Benzamid	2.17	8.69	2.18	100.5	3.27	2.02	93.2
	Creatinin	2.07	8.06	1.55	74.8	2.71	1.46	70.5

It is not thought that all the compounds used are actually present in soil. The substances were chosen rather to represent classes of compounds which conceivably might be in soils. Guanin, hypoxanthin, nucleic acid, peptone, and creatinin have been isolated from soil. It is

realized that the list is very incomplete. As opportunity to make or to purchase more compounds presents itself, the investigation will be considerably extended. From the data presented, it is observed that quite varying proportions of the pure proteins, which in reality are soluble in dilute alkali, are extracted. This seems to be a confirmation of the contention that the alkali extract as a whole does not represent a definite class of nitrogenous compounds. Of the simpler compounds, it is seen that the more acid and more closely neutral compounds are completely extracted and remain in the soluble nonprotein portion. An exception to this is found in the case of nucleic acid. This is to be expected from its tendency to combine with protein compounds to give insoluble nucleins. The action of the purin compounds is interesting. In general the more basic the compound the less the quantity recovered.

CONCLUSIONS

(1) If the results with the pure proteins be considered, it is probable that the alkali extract as a whole contains no definite group of compounds.

(2) From the results obtained by the precipitation of the alkali extract with trichloracetic acid it would seem that the soluble nonprotein fraction may contain most of the simpler nitrogenous compounds, and therefore its determination would give an index of the degree of decomposition of the organic matter in the soil.

LITERATURE CITED

(1) Bock, J. C., and Benedict, S. R.
1915. An examination of the Folin-Farmer method for the colorimetric estimation of nitrogen. *In* Jour. Biol. Chem., v. 20, no. 1, p. 47–59, 1 fig.

(2) Folin, Otto, and Denis, W.
1912. New methods for the determination of total non-protein nitrogen, urea and ammonia in blood. *In* Jour. Biol. Chem., v. 11, no. 5, p. 527–536.

(3) Greenwald, Isidor.
1915. The estimation of non-protein nitrogen in blood. *In* Jour. Biol. Chem., v. 21, no. 1, p. 61–68.

(4) Lipman, J. G.
1911. Microbiology of soil. *In* Marshall, C. E. Microbiology for Agricultural and Domestic Science Students. p. 226–291, fig. 66–72. Philadelphia.

(5) Potter, R. S., and Snyder, R. S.
1915. Amino-acid nitrogen of soil and the chemical groups of amino acids in the hydrolyzed soil and their humic acids. *In* Jour. Amer. Chem. Soc., v. 37, no. 9, p. 2219–2227.

(6) Schreiner, Oswald, and Shorey, E. C.
1910. Chemical nature of soil organic matter. U. S. Dept. Agr. Bur. Soils Bul. 74, 48 p., 1 pl.

(7) Shorey, E. C.
1913. Some organic soil constituents. U. S. Dept. Agr. Bur. Soils Bul. 88, 41 p., 1 pl.

(8) Van Slyke, D. D.
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino-acids. *In* Jour. Biol. Chem., v. 10, no. 1, p. 15–55, 2 fig.

OVIPOSITION OF MEGASTIGMUS SPERMOTROPHUS IN THE SEED OF DOUGLAS FIR

By J. M. MILLER,
Assistant in Forest Entomology, Bureau of Entomology

The larva of a seed chalcidid, *Megastigmus spermotrophus* Wachtl, has been very commonly recorded from the seeds of Douglas fir (*Pseudotsuga taxifolia*), but most of these records apply only to mature seed. The method by which the larvæ of this insect get into the seeds has not been previously described. The oviposition of the female, the period of the growth at which the seeds are infested, and the subsequent development of the larvæ are matters on which we have no published data.

The following is an account of the oviposition of this species observed at the Forest Insect Seed Station of the Bureau of Entomology at Ashland, Oreg., during the season of 1915.

During the season of 1914 a heavy emergence of adults of *Megastigmus spermotrophus* from Douglas fir seed of the 1913 crop occurred in the vicinity of Ashland. From stored seed kept in a rearing box the male adults began to emerge on April 12, and the females on April 16; 2,897 adults emerged from 6¾ ounces of seed, the period of maximum emergence occurring between April 23 and May 11. A number of these adults were liberated in a small cage kept in the laboratory. It was found that the adults would not live any length of time unless fed. Pieces of blotting paper saturated with sugar solution were hung in the cage and on this the adults were frequently seen feeding. Young Douglas fir cones were kept in the cage with the adults for a period of about three weeks; and although copulation was frequently observed, no attempts were noted on the part of the females to oviposit in the cones.

During the season of 1915 another effort to secure a record of oviposition in a rearing cage met with far better success. Various lots of infested Douglas fir seed were kept at the station, and the emergence of the adults from this seed was quite similar to that observed in 1914. The maximum period of emergence in the laboratory occurred between April 20 and May 2. From cones which were kept caged over winter under outdoor conditions at the same elevation, the maximum emergence occurred between May 1 and 16. At elevations of 3,000 to 4,000 feet the emergence occurred during the latter part of May, and above 4,000 feet much of the emergence occurred in June.

Many adult chalcidids were liberated at different dates between April 18 and May 20 in a cage considerably larger than that used in 1914 (Pl. V,

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
da

Vol. VI, No. 2
Apr. 10, 1916
K—28

fig. 1). This was kept outdoors in a partially shaded position. The adults were fed as before with sugar solution.

A Douglas fir branch bearing cones about 3 weeks old was placed in this cage on April 18. The young cones were then about 1½ inches long, the scales were still soft, and the seeds had the milky interior and unhardened coat. The base of the branch was kept in a jar full of moist earth. Fresh branches were placed in the cage at intervals until May 15, when the cones were estimated to be about half mature.

Mating was observed in this cage and in the emergence vials of the rearing boxes during the entire period. The first oviposition of a female on a cone was observed on April 20 at about 3.30 p. m. A female was observed crawling about over the bracts and feeling the scales with her antennæ. This lasted for several minutes; then the female paused on one of the exposed scales with her head pointed toward the base of the cone. After resting quietly for a moment the abdomen was lifted and at the same time the posterior end was doubled under so that the sheath of the ovipositor was brought forward between the legs until the tip rested on the surface of the cone scale at a point directly under the insect's head. The point chosen for the insertion of the ovipositor was close to the outer edge of the scale on which the female rested. The sheath of the ovipositor was then withdrawn and assumed its normal position back of the abdomen, while the ovipositor was slowly forced down into the cone. The abdomen was gradually lowered as the ovipositor was thrust into the cone until finally the entire body rested close to and in a line parallel with the surface of the cone scale (Pl. VI, fig. 3). In this position the female rested for about a minute and then withdrew the ovipositor. This was accomplished by raising the body and doubling the abdomen until it assumed a position similar to that in which the oviposition was started (Pl. VII, fig. 1). This allowed the ovipositor to be withdrawn and returned to its sheath.

The oviposition of two females was recorded on April 22 and that of the same number on April 23. Between this date and April 26 no oviposition and very little activity on the part of the seed chalcidids were observed. On the morning of April 26 a female was observed ovipositing, and this operation was recorded four times during the day. On April 27 about the same activity occurred. April 28 was a warm, sunny day and great activity on the part of the females occurred. The cage at this date contained 10 cones and about 50 females. At almost any hour during the day from one to three females could be seen either ovipositing on the cones or preparing to do so. From April 29 to May 2 cool rainy weather prevailed, and almost no activity on the part of the chalcidids occurred in the cage. May 3, 4, and 5 were warm, sunny days, and the oviposition could be witnessed at any time during the day. Oviposition in the 10 cones in the cage on these dates was in progress continuously during the day, at which time the best observations of the act were obtained.

A spell of rainy weather persisted from May 8 to 25, and no further records were secured. The subsidence of emergence after the latter date made it impossible to obtain adults for liberating in the rearing cages, and efforts to secure further records were not attempted.

Difficulty was encountered in securing photographs, as females will not oviposit if even slightly disturbed. If a cone was jarred in any way while a female was in the act, the ovipositor would be withdrawn as rapidly as possible. Even though the ovipositor was inserted deep in the cone the female would struggle to disengage it and fly away. However, by raising the glass on the front of the cage it was possible to focus a camera directly on the cones, and several pictures were obtained in this way. For the purpose of further study and dissection, a number of females were captured and killed with the ovipositor thrust into the cones. This was best accomplished by quickly immersing the cone on which the female rested in a graduate filled with chloroform. This killed the female so quickly that her efforts to withdraw the ovipositor were seldom successful. Several of the females which were killed in this position were photographed (Pl. VI, fig. 1, 2).

The time required for oviposition varies from two to five minutes. The same female was observed to oviposit five times on the same cone, and it is probable that the operation is repeated many times before the egg-laying capacity is exhausted. The point selected for the insertion of the ovipositor was always on the surface of a scale, never on a bract, and may be either on the margin or near the center of the scale. The female always assumed a position with head pointed toward the base of the cone. As Douglas fir cones were pendent at the time of oviposition, this allowed the female to stand with her head pointed upward (Pl. V, fig. 2, 3).

In cones which were dissected with the ovipositor of the female inserted it was found that the ovipositor reached the seed in a few cases only. Apparently where successful the ovipositor passes through the scale nearest the surface and underlying bracts until it reaches the second or third scale from the surface. It then follows down through the center of the last scale nearly to its base and then turns forward into the seed just ahead of it (Pl. VII, fig. 2, 3). The fact that the ovipositor was seldom found in the seed in the cones dissected is doubtless due to the fact that the female partly withdrew her ovipositor in the death struggle.

It would seem that successful oviposition occurs only when the egg is deposited in the seed, as the larvæ have never been found to work their way through the tissues of the cone, and their development is confined entirely to the interior of one seed.

Numerous cases were found in which the ovipositor did not penetrate even as far as the base of the scale. This occurred most frequently where the cones were of such an age that the scales had hardened. In these cases the tough tissues of the scales seem to bend the ovipositor out of

its course, and in a number of the dissections the ovipositor was bent and twisted around to a course directly opposite to that intended. This condition was not encountered where the cones were still young and soft. In fact, after the cones become hardened it is difficult to realize how they can become infested at all by the chalcidids.

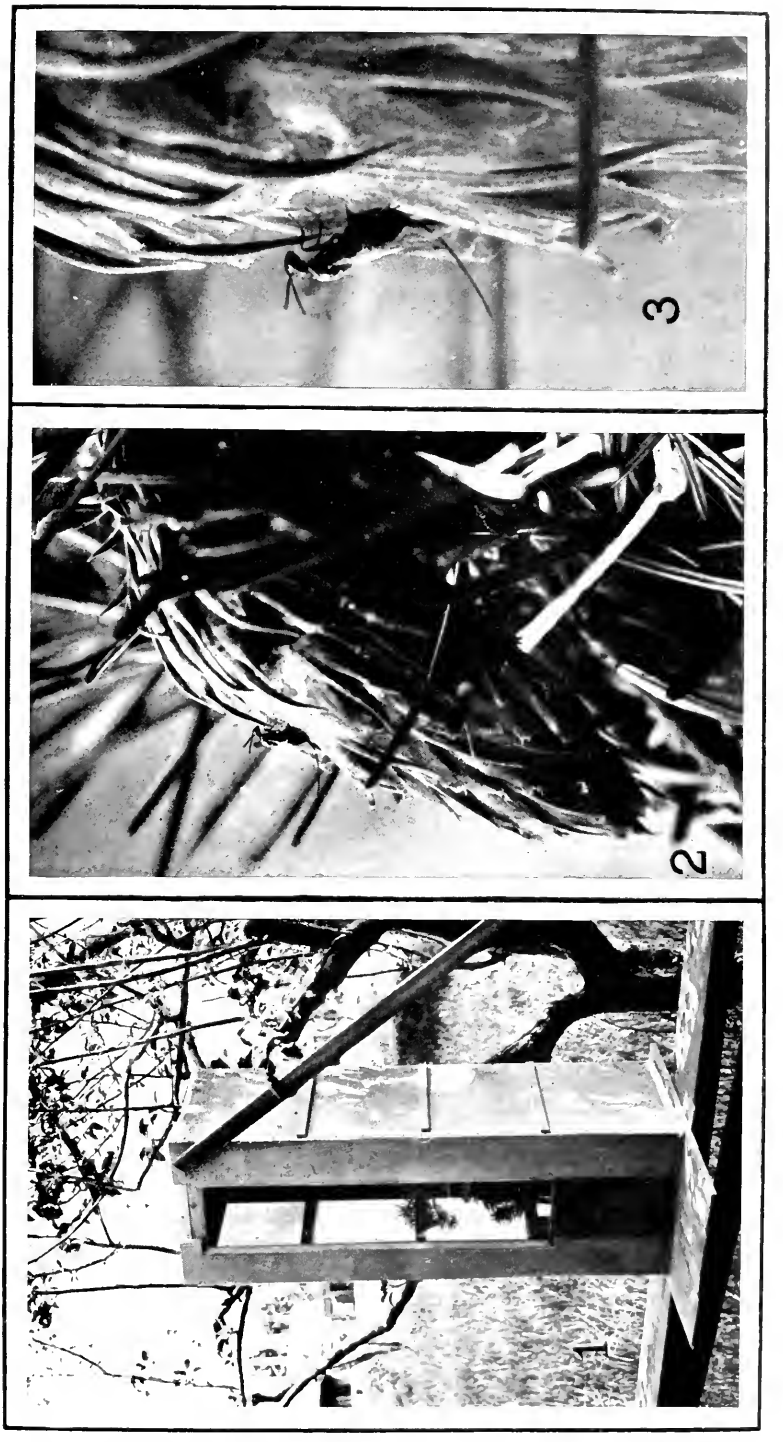
Actual oviposition in the field by the seed chalcidids was observed only once—on May 28 by Entomological Ranger J. E. Patterson. While collecting cones he noted a female on one cone with her ovipositor inserted. The insect withdrew the ovipositor and left the cone very soon after it was noticed.

PLATE V

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1.—Type of cage in which the oviposition of *Megastigmus spermotrophus* was observed. This cage was kept under outdoor conditions. A branch bearing young cones of Douglas fir was set in a jar of moist soil and kept in the cage with the females.

Fig. 2, 3.—Female resting on cone with ovipositor inserted. Photographed from life. On left, original; on right, enlargement of same to show exact attitude of the female.


3
2
1

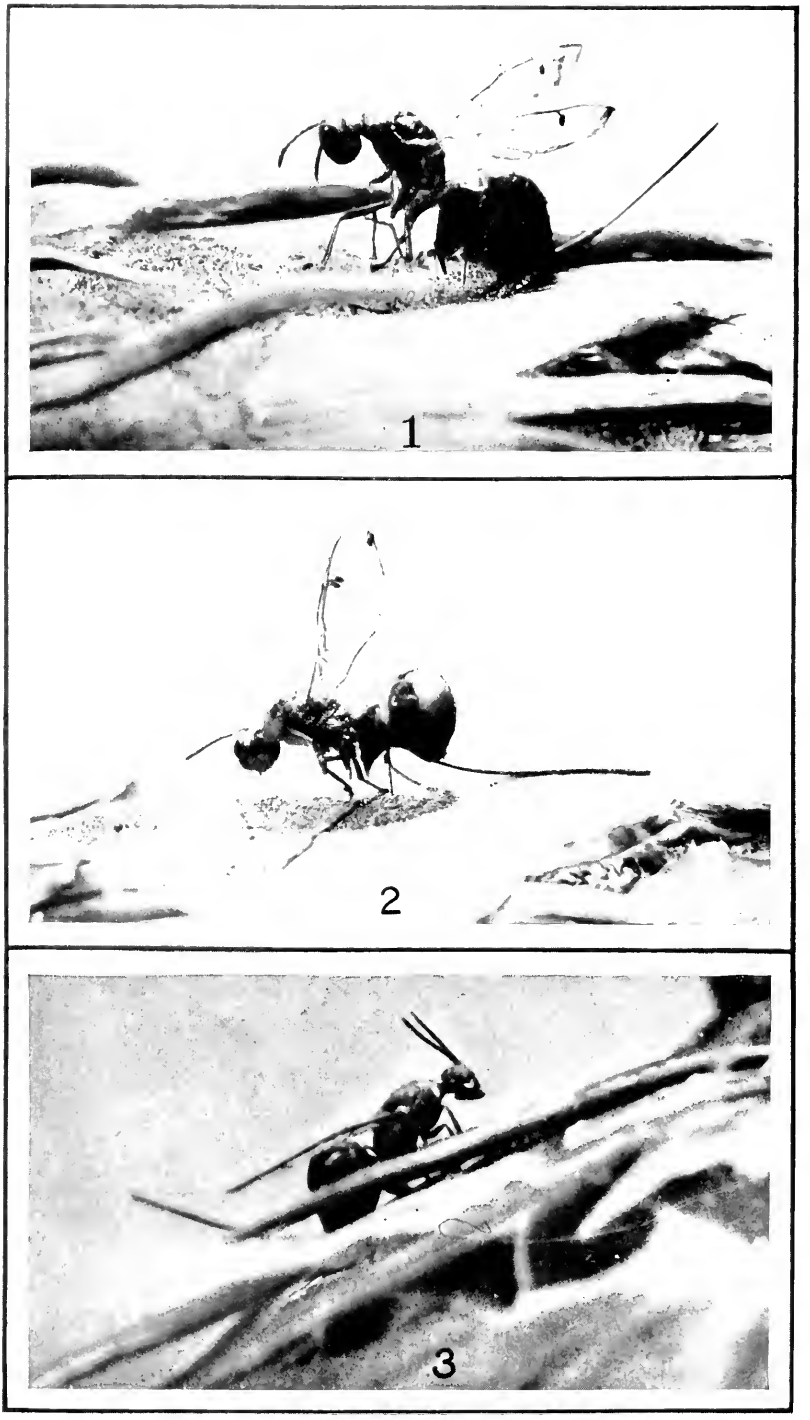

1
2
3

PLATE VI

Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

Fig. 1, 2.—Two positions of female on surface of cone with ovipositor inserted. Photographed from dead females which had been killed in this position. Enlarged.

Fig. 3.—Female resting on cone with ovipositor inserted. Photographed from life. Enlarged.

PLATE VII

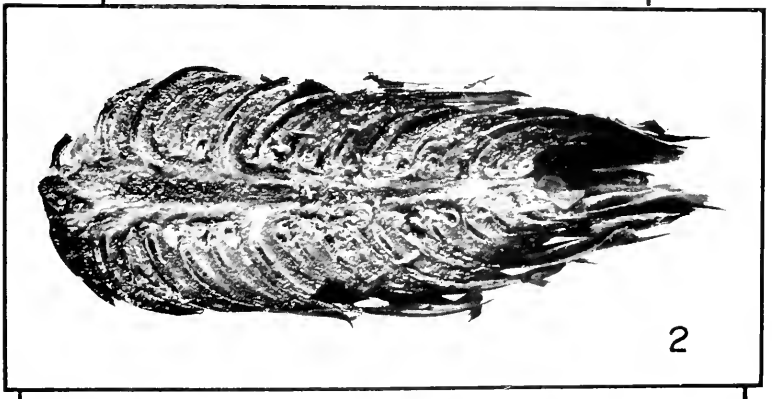
Oviposition of *Megastigmus spermotrophus* in the cones of Douglas fir:

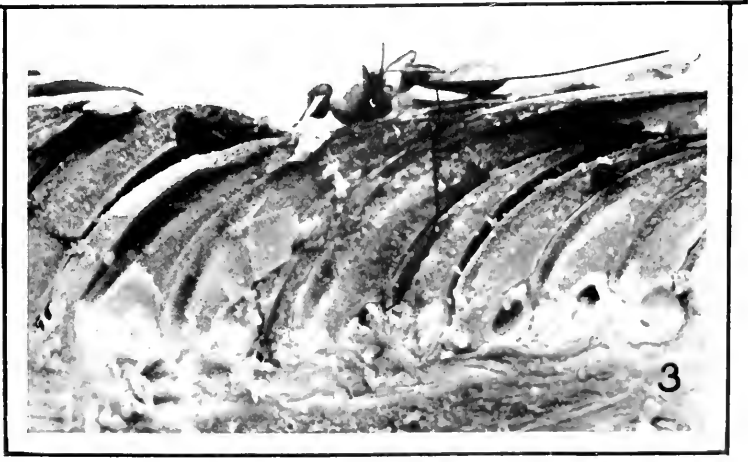
Fig. 1.—Female in act of withdrawing ovipositor from cone. Photographed from life. Enlarged.

Fig. 2.—Section through a Douglas fir cone on which a female has been killed while in the act of ovipositing.

Fig. 3.—A portion of same cone and dead female with ovipositor inserted. Slightly retouched to show course followed by ovipositor in reaching the seed.


1


2


3

CITRUS CANKER[1]

By FREDERICK A. WOLF,[2]
Plant Pathologist, Alabama Agricultural Experiment Station

INTRODUCTION

The ravages of certain insect pests and plant maladies have, in a considerable number of instances, been so severe as to cause intense alarm. It has been feared in the case of several crops that their culture was no longer possible in certain sections because effective means of preventing the losses resulting from such ravages were not then known. Within the last two years it has been realized that a new disease known as Citrus canker has been introduced into the Citrus-growing sections of the Gulf Coast States. This disease, beyond all doubt, is the most destructive malady affecting species of Citrus, and when it was realized that its control and eradication were so difficult, alarm concerning the future production of Citrus fruits became almost an hysteria. Those who have never seen Citrus canker under field conditions regard the reports of the highly infectious nature of this disease, of its destructiveness, and of the difficulties experienced in its eradication as the results of an overwrought imagination. The severity of Citrus canker has not been exaggerated, however, and growers should lose no time in preventing its further dissemination and in effecting its eradication.

HOSTS OF THE ORGANISM

Citrus canker has been found to affect many of the varieties and species of Citrus, and in all probability none of the species of this genus are entirely immune. It is perhaps productive of more serious injury to the varieties of grapefruit, or pomelo (*Citrus decumana*), than to any other of the Citrus fruits. Seedling grapefruits appear to be more susceptible to canker than the budded varieties. Some regard the injury to the hardy or trifoliate orange (*Citrus trifoliata*), which is extensively used as the stock upon which to bud other species of Citrus, as equally severe. Certain of the varieties of round oranges (*Citrus aurantium*) are known to be very susceptible to Citrus canker and under favorable conditions suffer as severe injury as grapefruits. The disease occurs also on varieties of the sweet orange. Oranges of the mandarin group (*Citrus nobilis*),

[1] Published with the permission of the Director of the Alabama Experiment Station.

[2] The writer is greatly indebted to his colleague, Dr. J. S. Caldwell, for suggestions and material aid during the progress of this investigation and for assistance in the preparation of the manuscript. Much of the chemical portion of the investigation would have been impossible but for the skillful and arduous assistance of Messrs. A. C. Foster and C. W. Culpepper, formerly laboratory aids in the Department of Botany of the Alabama Polytechnic Institute. To each of these gentlemen grateful appreciation for the several services is hereby acknowledged.

including mandarins, tangerines, and Satsumas, have also been found to be diseased. The disease has been observed, too, on several varieties of lemons (*Citrus medica*) and limes (*Citrus limetta*). Thus far Citrus canker in Alabama has not been found to attack kumquats, the four species of which Swingle (17) [1] regards as belonging to the genus Fortunella. It has been observed, however, on the leaves and twigs of the kumquat in Louisiana. Swingle (18) reports its occurrence on this host in Japan.

HISTORY OF THE DISEASE

Citrus canker is not of American origin, but beyond doubt was introduced into the Gulf States from Japan. This statement is supported by the fact that it is known to occur in Japan and the Philippine Islands (18), and, so far as can be learned, it appeared in the United States several years ago simultaneously with the importation of Satsuma and trifoliate stock into Texas in order to supply the large demand for trees for Citrus plantings. Whether it is indigenous to Japan is not known, but it is probably native of parts of eastern Asia. Since its introduction into Texas it has been disseminated by the shipment of diseased trees to other States and has further been introduced by shipments to these States direct from the Orient, so that it now occurs in parts of Florida, Alabama, Mississippi, Louisiana, and Texas.

Citrus canker had probably been present in the United States for five or six years before it was recognized as a new Citrus disease. Specimens were first collected in September, 1912, but it was not until July of the following year (1) that the Office of Nursery Inspection of Florida realized that these specimens did not represent an unusual manifestation of scab caused by *Cladosporium citri*. This mistake in diagnosis had also been made by inspectors in other Gulf States and by officers of State Experiment Stations and of the Federal Department of Agriculture. Japanese authorities had also mistaken this disease, since specimens received at the Florida Agricultural Experiment Station (2) from Japan had been identified as scab. The disease was brought to the writer's attention in February, 1914, and has been interruptedly studied by him since that time.[2]

A number of publications upon Citrus canker, all preliminary in nature, have appeared. These papers call attention to the presence of the disease in the several States, briefly describe its appearance, and recommend concerted cooperation in its eradication. The disease was first regarded as of fungoid origin, and the first claim that bacteria are the primary cause of the disease was made by Hasse (6). The present publi-

[1] Reference is made by number to "Literature cited," pp. 98–99.

[2] The writer severed his connection with the Alabama Agricultural Experiment Station on January 1, 1916. This study therefore is incomplete, time not having been afforded for verification of all portions of the study, and certain problems which have appeared in connection with the work have not been investigated. However, it was deemed advisable to record the results of the studies thus far conducted.

cation has for its purpose the recording of studies which are in part confirmatory of previous studies (2, 5, 6, 16, 19) and which further contribute to our knowledge of this disease.

ECONOMIC IMPORTANCE

The serious nature and unusual virulence of Citrus canker and the jeopardy in which it has placed the Citrus industry can best be realized when it is recalled that the Federal Horticultural Board, on January 1, 1915, placed a quarantine on the importation from all foreign countries of Citrus nursery stock, including buds, scions, and seeds, in order to prevent further introduction of the disease into the United States. It is difficult to obtain figures as to the number of nursery and orchard trees which have been destroyed in an effort to eradicate the disease from the Gulf coast. It is equally difficult to obtain accurate figures on the amount of money which has already been expended by the Federal Government, together with the State horticultural boards, liberally aided by various organizations and by private subscriptions, in an effort to stamp out Citrus canker. Suffice it to say that the actual cost in money for eradication and for trees destroyed has been enormous.

SYMPTOMS OF THE DISEASE

Citrus canker affects the leaves, twigs, larger branches, and fruits in a characteristic manner. Upon any of these parts the diseased areas are light brown in color and project more or less above the surrounding tissues. The cankerous areas consist of a corky mass of cells covered by a lacerated grayish membrane. It can be determined with certainty without a microscopic examination in case one has typical diseased material, and in case one has seen the disease in the various stages of development under field conditions. It is sometimes impossible to be certain whether meager specimens such as are sometimes sent in for identification are affected with canker or with some other leaf trouble. This is especially true in the case of canker on the Satsuma orange. If, however, one is permitted to make a field examination, and can thus learn of the origin of the trees, and can also observe adjacent trees, typical material may be found if Citrus canker is present.

OCCURRENCE ON THE LEAVES

The first evidence of canker on the leaves is the appearance of very small oily or watery dots on the lower leaf surface. They may appear on either surface, but are more commonly found on the lower leaf surface. They are of a darker green color than the surrounding leaf tissue and may at this stage be mistaken for oil glands (fig. 1, 2). The diseased areas are slightly convex, however, and within a few days will have extended through the leaf, appearing on the upper surface as

greenish yellow spots. By continued development the convex surface of the spots comes to be more and more elevated until the epidermis is broken by the increased tension and the subjacent tissues are thus exposed to desiccation. The exposed tissues then become corky, darkening with age. The ruptured epidermis is turned back irregularly and persists as a lacerated membrane. The margin of the diseased area maintains an oily appearance even after the spots have ceased to increase in size. Mature spots (Pl. XI, fig. 1) vary in size from very minute to a quarter of an inch in diameter and are typically circular in outline. They may occur singly; or when they are very numerous, fuse, thus forming large, irregular areas. Cankered areas are typically elevated on both leaf surfaces. In the case of canker on Satsumas (Pl. VIII, fig. 2, and Pl. IX, fig. 4), however, there is little or no elevation of the upper leaf surface. Neither is the oily margin so evident on this host, especially in case of old cankers, in which diseased tissues have become dark brown, simulating the appearance of melanose. The appearance of the disease on leaves of *Citrus trifoliata* as shown in Plate X, figure 1, is very similar to that on grapefruit. Stevens (2) reports that he has never found Citrus canker on trifoliate orange leaves. The uninvaded tissues surrounding the cankers are paler green than the normal tissue and gradually form a chlorotic or yellowish zone (Pl. VIII, fig. 1, and Pl. X, fig. 6), which may invade all the tissues not actually occupied by the cankers. At this stage considerable defoliation, especially in the case of grapefruit and trifoliate oranges, may occur. Cankers on the leaf petioles cause defoliation even though the leaves are otherwise uninvaded.

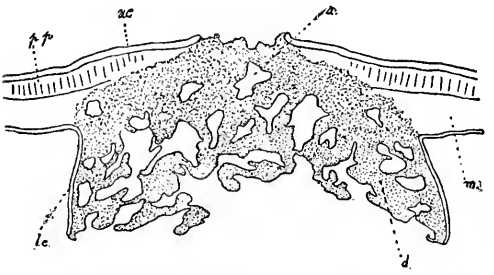


FIG. 1.—Diagrammatic representation of young open type of Citrus canker of half the diameter of the one shown in figure 2. *pp*, Palisade parenchyma; *ue*, upper epidermis; *le*, lower epidermis; *d*, diseased tissues: *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

OCCURRENCE ON THE TWIGS AND BRANCHES

Limb canker appears more commonly on very young twigs because of the absence of any considerable suberization, but larger branches are subject to infection. Growing cankers have been observed on limbs ½ to ¾ inch in diameter (Pl. VIII, fig. 3, 4). The disease has been found on branches of grapefruit, trifoliate oranges, lemons, Satsumas, and certain varieties of round oranges. Cankers on twigs are first apparent as small, circular, watery spots. They rapidly enlarge, become blisterlike and the epidermis ruptures, exposing the cankerous tissue below. At this stage they project more or less prominently and are very similar

in appearance to the spots on the foliage. Isolated cankers remain circular in outline. When the spots originate close together, however, large irregular, variously cracked or fissured cankers are developed, which may involve an area several inches in length. The epidermis persists as a grayish broken membrane at the margin of these cankers (Pl. VIII, fig. 5). Twigs and larger branches may be completely girdled, resulting in the death of the distal parts. Affected trees exhibit a stunted growth, and numerous branches may be developed below the dying tips.

The disease is very severe upon stems of grapefruit and trifoliate oranges. On the latter host the thorns are abundantly cankered and the base of the thorns appears commonly to be the initial seat of infection.

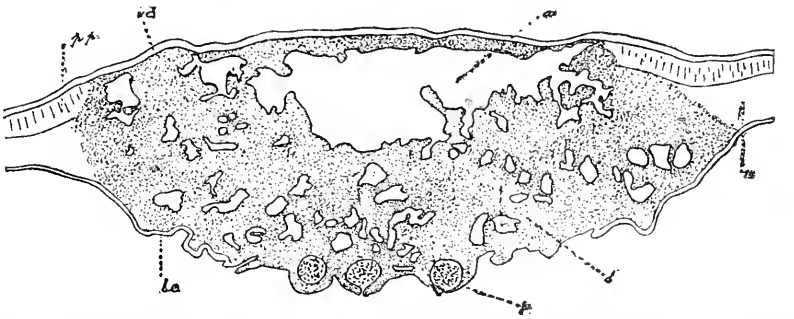


FIG. 2.—Diagrammatic representation of canker on old Citrus leaf: *pp*, Palisade parenchyma; *uc*, upper epidermis; *le*, lower epidermis; *p*, pycnidium of *Phoma socia;* *d*, diseased tissues; *a*, air space arising from tensions due to the enlargement of cells and disintegration of tissues.

Limb cankers on trifoliate oranges oftentimes are zonate with different shades of brown, especially if the outer membranes have not yet been ruptured.

OCCURRENCE ON THE FRUIT

The cankerous areas on the fruits are quite similar in appearance to the leaf cankers, differing mainly in the larger size of the former. They are scurfy elevations, for the most part circular in outline and surrounded by a zone of chlorotic rind tissues. The corky diseased tissues are quite superficial; and if the spots unite, large scaly areas are formed (Pl. X, fig. 2). In this case the fruits may crack open because of their increase in size owing to the growth of the fruits and may become prematurely yellow and drop. Fruits which are badly cankered and have burst open are, of course, subject to invasion by various organisms of decay. Even if they remain on the tree, they are rendered very unsightly and are unsalable.

OCCURRENCE ON THE BUDS

Nurserymen experience considerable losses from failure of Citrus buds to unite with the stock. In some cases when *Citrus trifoliata* seedlings affected with canker are used as stock, losses of over 50 per cent have

been sustained. The operation of budding either directly conveys the organisms into the wounded tissues or they are subsequently washed into them from cankers above the insertion of the bud before union has been effected.

ETIOLOGY OF THE DISEASE

The primary cause of Citrus canker is a bacterial parasite, *Pseudomonas citri* Hasse (6). Hasse isolated this organism from cankers on grapefruit and proved it to be pathogenic to grapefruit seedlings. This claim was established at a time when the disease was regarded as of fungus origin. Hasse further pointed out the fact that a number of fungi were isolated from old Citrus cankers. The writer had found a fungus, as had also Prof. H. E. Stevens, of the Florida Agricultural Experiment Station, belonging to the form genus Phoma, commonly associated with cankerous tissues. The writer's initial inoculations were made not with pure cultures of Phoma, as has subsequently been learned, but with cultures which had overrun the bacterial parasite. Successful infections reported in the previous publication (19) are thus accounted for. Consideration will be given in another part of the present report to the part which *Phoma* spp. and certain other fungi play in the production of Citrus canker.

PATHOGENICITY

Pseudomonas citri has repeatedly been isolated during the past season from cankers on grapefruit, trifoliate orange, lemon, and Satsuma oranges. The strains from these different hosts present the same cultural characters. Because of this, together with the added fact that no difficulty has been experienced in making cross inoculations, the strains are regarded as identical.

The plants used in making the inoculation experiments were grown in the greenhouse at Auburn, Ala. Typical cankers have been produced on McCarty and seedling grapefruits (Pl. IX, figs. 1, 2), pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. Infections on all these species were as readily secured, whether the organism had been isolated from *Citrus trifoliata*, Satsuma, grapefruit, or lemon. Neither was there any evident difference in virulence of any of the strains. A suspension of the organism taken from pure cultures grown either on potato cylinders or in bouillon was used in making the inoculations. This suspension when applied with an atomizer resulted in a high percentage of successful inoculations. A greater number of successful inoculations were secured, as would be expected, when the plants were covered with bell jars to prevent the too rapid evaporation of the moisture. When the inoculum was introduced into the tissues of leaves, stems, or fruits through needle punctures, cankers developed in all cases. In some cases the suspension was applied to leaves with the fingers. They were dipped into the suspension

and the material was then applied by gently rubbing the leaves between the thumb and fingers. In a few cases it was arranged so that twigs bearing young leaves could be immersed for an hour or two in a bacterial suspension. Leaves inoculated in this manner are shown in Plate VIII, figure 2. It is to be noted that the infections are so numerous as to involve the greater part of the lower leaf surface.

The period of incubation appears to vary, depending on temperature, moisture, and age of the plant tissues. Very definite signs of the disease have been noted within 72 hours after inoculation. In other cases 10 days were required before the infections were evident to the eye. The longest periods were secured on Satsumas.

An organism of the same color as *Pseudomonas citri* and similar in appearance on certain media, but which does not exhibit the characteristic growth of *P. citri* on potato cylinders, has commonly been isolated from old cankers. This organism has not been found to be pathogenic on species of Citrus, however. There can be little doubt of the pathogenicity of the organism concerning which Hasse made her preliminary report (6). It is to be noted that her Plate X, figures *A*, *B*, represent natural infections and Plate X, figure *C*, artificially produced cankers. These, however, are regarded as identical in appearance. Artificially inoculated seedlings are represented also in Plate IX. As can readily be seen, the artificial cankers are much more prominently projecting than natural ones, are evidently greenish white in color, and there has been no discoloration of the leaf tissue surrounding the spot. The writer has never, under field conditions, seen specimens which resembled these artificial inoculations represented in Hasse's Plates IX and X, and he, furthermore, has examined fresh specimens in various stages of development sent from Florida, Alabama, Mississippi, Louisiana, and Texas. However, cankers similar in appearance to Hasse's artificial cankers have been produced in the greenhouse. Following her suggestion that the open, spongy type of canker is due to favorable conditions of moisture and temperature, seedling grapefruit which had been atomized with a suspension of *P. citri* were kept continuously covered with a bell jar. They were watered sufficiently often so that the air under the bell jar was maintained at a high relative humidity. Within 10 days the cankers shown in Plate IX, figure 3, had developed. These are regarded as similar in appearance to those previously produced by Hasse and represented in her Plates IX and X.

DESCRIPTION OF PSEUDOMONAS CITRI

The primary cause of Citrus canker is a yellow, 1-flagellate organism. Its motility can be observed when taken directly from young cankers and examined in a drop of water. In this case it will be found to occur singly or in pairs. On solid media it may form into chains of six or more elements. It is quite variable in shape and size. When taken

from young cankerous tissues it is usually a short rod with rounded ends which measures from 1.5 to 2.5 by 0.5 to 0.75μ. In old cultures the elements may be ellipsoidal. No endospores have been demonstrated; nor have involution forms been observed.

The organism stains readily with solutions of carbol fuchsin, analine gentian violet, and methylene blue. Only negative results have been secured with Gram's stain. When the organism has been grown on potato cylinders and is stained with anilin gentian violet, it has an apparent capsular portion (fig. 3). This capsular portion gives rise, no doubt, to the viscidity which characterizes its growth on steamed potatoes. The slime on old potato cultures can be drawn out an inch or two and does not dissolve readily in liquid cultures.

Young cultures of this organism on steamed potato cylinders have a very characteristic appearance. The growth is bright yellow, smooth, moist, glistening, and raised, with a narrow white zone along the margin of the bacterial growth. This white margin does not persist, since by its rapid growth the organism covers the entire surface of the medium. It acts very strongly on potato starch, as indicated by the entire absence of an iodin reaction on steamed potato cylinders 6 to 8 weeks old. The middle lamellæ in such old cultures have been dissolved, and the empty cells can readily be separated from one another.

FIG. 3.—*Pseudomonas citri*: *a*, Stained with carbol fuchsin; *b*, stained with Williams's flagellar stain (adapted from Hasse); *c*, stained with anilin gentian violet.

The organism has been grown on nutrient agar made by adding a water extract of corn meal, bean meal, green beans, cowpeas, potatoes, rice, orange juice, or orange leaves and stems, but the growth on none of these media is characteristic. No attempt was made to titrate any of these media to determine their acidity or alkalinity.

Colonies appear on the second day in poured plates of green-bean agar kept at room temperature. Within four or five days the surface colonies in poured plates will have become 2 or 3 mm. in diameter. The margin of the colonies is entire, and they are opaque yellow in color. They are appreciably raised and have a smooth, wet-shining surface. The character of the margin and of the surface is shown in Plate XI, figure 4. It will be noted that the reflection of the two windows in the room in which the exposure was made is shown in each of the colonies.

A filiform growth, following the line of the stroke and widening at the base of the slant, is formed in stroke cultures on green-bean agar. The growth does not penetrate the agar and does not give rise to the production of any stain or odor. In stab cultures on this medium a filiform but otherwise nontypical growth is produced, which when viewed from

above appears like the surface colonies in poured plates. Growth in stab cultures on various media is always or nearly always best at the surface of the media.

On nutrient-gelatin plates the colonies are circular in outline, slightly raised, entire margined, and yellowish. In gelatin stabs a filiform growth appears along the line of puncture, with the greatest growth at the surface of the medium, and with a rather slow liquefaction.

The organism is regarded as a facultative anaerobe. No gas is formed in fermentation tubes containing a 2 per cent solution of Witte's peptone. With this as a basal solution, five solutions were made by adding 1 per cent of one of the following carbon compounds: Saccharose, dextrose, lactose, maltose, and glycerin. All inoculated tubes developed a slight cloudiness, which extended into the closed end of the tube by the second day. More vigorous growth occurs in the open end of the tube, however, and after four or five days the cloudiness is very marked. Yellowish flocculent particles appear later in the open end, and a yellowish ring is formed at the surface. No gas formed in any of the solid media in which the above-mentioned carbon compounds were added to the nutrient agar.

In stab cultures on litmus-dextrose, litmus-lactose, litmus-saccharose, and litmus-glycerin agar no gas formation was apparent in 10-day old cultures. It is not known whether acidification will occur in old cultures on these media.

In sterile tubes of litmus milk there is a rather slow reduction of the litmus. After five days there is a slight increase in the blue color. The reddish whey is gradually formed on the surface, and the casein is precipitated.

There is no reduction of nitrates in Witte's peptone solution containing a trace of potassium nitrate. Phenoldisulphonic acid was used as a reagent 10 days after the date of inoculation, at which time both the check and the solution in which the organism was growing were colorimetrically alike.

Only negative tests for indol were secured in peptonized beef-bouillon cultures. A very conspicuous clouding occurs in this medium within 24 hours after inoculation. As these cultures get older they become somewhat flocculent, and a yellowish ring is formed at the surface of the media.

The thermal death point, as found in preliminary tests, was between 58° and 70° C. In order to determine more nearly the point, tests were made by exposing the organism taken from potato cylinder cultures, and transferred to tubes of bouillon. The tubes were then placed in a water bath for 10 minutes at some given temperature between these limits. The temperature of the bath was kept constant during the period of exposure. The tubes were subjected to room temperature for several

days to observe the development of cloudiness. In order to be certain, however, of the viability of the organism, loops of bouillon from these tubes were transferred to planted plates of nutrient agar, and the subsequent development noted. No growth occurred in the tubes exposed at temperatures above 65° C.

No attempts have been made to determine the exact degree of tolerance of this organism to acids. When transfers were made to dextrose-peptone agar +10, +20, and +40 Fuller's scale, it was found at the end of three days to have grown in the first two, but growth was completely inhibited in +40 acid. Hydrochloric and citric acids were employed in acidification.

The organism seems to exhibit a very considerable resistance to drying. In the desiccation experiments bacteria from vigorous pure cultures on potato plugs were smeared by means of a sterile platinum needle on clean miscroscopic slides in moist chambers. The moist chambers containing the microscopic slides were sterilized prior to transferring the bacterial smear to the slides. These preparations were made on June 1, and placed in a wall closet in the laboratory. On July 1, August 1, and September 1 several of the microscopic slides were removed from the moist chambers and placed in sterilized Petri dishes, using proper aseptic precautions in making the transfers. Tubes of melted nutrient agar which had been cooled almost to the point of solidification were poured upon these smeared slides. No growth occurred in the case of those tested on September 1, but those tested on July 1 and August 1 were still alive. From this it is believed that the organism can retain its viability for about two months.

The group number according to the descriptive chart of the Society of American Bacteriologists is 221.3332513.

LIFE HISTORY OF THE ORGANISM

Pseudomonas citri, so far as is known, passes its entire life cycle under natural conditions within the tissues of the host. New infections appear in spring shortly after the new growth has begun. In southern Alabama the first appearance of Citrus canker in the field was noted on May 11, in 1914, and on May 27, in 1915. Old diseased areas on the foliage together with the cankers on the twigs and larger limbs are undoubtedly the source of infection in the spring. New leaves formed near old twig cankers are especially liable to become diseased first. Infections are not confined to the new growth, however. Old diseased areas on leaves and branches may enlarge by the renewed growth of the organism which has remained dormant on the margin of the old cankers. New cankers may also develop on old foliage and twigs, especially near the old, actively growing cankers. Under favorable conditions new infections may appear at any time throughout the growing season of the host. In one instance

new infections are known to have appeared abundantly under field conditions during November, 1914. Old leaves on the ground may possibly harbor the organism and there it may remain viable for a long time. Unsuccessful attempts, however, have been made to recover the organism from leaves kept in the laboratory from September, 1914, to May, 1915; nor has recovery been possible in the case of twig cankers kept under laboratory conditions from March to October, 1915.

It is believed, moreover, that the organism survives the winter in fallen leaves and that these fallen leaves constitute a very important source of infection in the following spring, especially in the case of nursery trees which have been planted between diseased grove trees.

There is every reason to believe also that the organism can remain alive in soil. This is evidenced by numerous instances in which new sprouts have come up from the roots of diseased trees which had been burned. A large percentage of these sprouts are early found to be diseased. Furthermore, the leaves on the lowermost branches or those in actual contact with the soil are commonly the first to become diseased.

The fact that the stomata, or breathing pores, on species of Citrus occur only on the lower leaf surfaces and that infections developed only on the lower surface of the leaves in all of the inoculation experiments in which the plants had been sprayed with bacterial suspensions led to the inference that the canker organism must gain entrance to the leaves through the stomata. That such is the case was established by leaf sections which were fixed 72 hours after inoculation and which were subsequently properly infiltrated, cut, and stained (fig. 4). Lenticels very probably serve as portals of entrance for the organism into the stems. A film of moisture on the surface of the leaf, twig, or fruit enables the organism to move about and thus to gain entrance into the substomatal cavity. Under ordinary conditions inoculation will be successful only in the presence of moisture. Wounds or abrasions from any cause may afford an entrance to the bacteria. Inoculations not infrequently occur through wounds made by thorns. Inoculations on leaves made by thorn scratches are shown on Plate X figure 3. Thorns which come in contact with limbs near by may inflict wounds which have subsequently been observed to be the point of origin of limb cankers. Cankers have also been found at the point of contact of limbs which rub together through movement by the wind.

When once the bacteria have passed through the stomata into the substomatal cavities, they multiply rapidly and effect a passage between the host cells to the intercellular spaces which become filled with solid masses of bacteria. As the bacteria continue to multiply, the cells farther away from the substomatal chambers become involved seriatim. In this way an area circular in outline and extending entirely through the leaf comes to be invaded. Various stages of invasion of the leaf tissues have

been observed in serial paraffin sections. Within three to five days after inoculation the disease is evident in the form of oily or watery spots. Within another week with favorable weather conditions and on young leaves the epidermis will have ruptured on one or both surfaces and open cankers will have formed. At this stage, before the exposed cells have become desiccated, the greatest danger of spreading the infection exists. Young tender tissues seem to be more susceptible to infection at this time than mature tissues. The disease progresses more rapidly, too, in young tissues than in older parts.

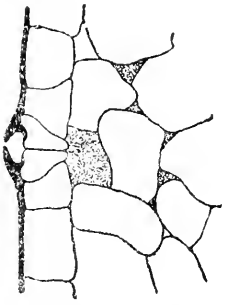


FIG. 4.—Early stage of Citrus canker in cross section on a young leaf of seedling grapefruit. The leaf was inoculated by immersion in a suspension of *Pseudomonas citri* from pure culture. The material was collected 72 hours after inoculation. It was then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. The organism entered the leaf through the stoma, multiplied in the substomatal chamber, and spread to adjacent intercellular spaces. Drawing made with a camera lucida. × 600.

RELATION BETWEEN PARASITE AND HOST

No study has been made other than the preliminary account of Hasse (6) of the effects of *Pseudomonas citri* on Citrus tissues. She states (p. 98) that—

> There is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis. The cells are found to be filled with short rod bacteria. All the cells exhibit more or less enlargement. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized.

This problem was first attacked by making a histological study of the diseased tissues. For this purpose cankers in various stages of development on fruits and leaves were cut out so as to include some of the surrounding healthy tissue. Cankers which had developed under conditions of very high relative humidity (Pl. IX, fig. 3, and Pl. X, fig. 4) and which were consequently of the spongy type and white in color yielded especially interesting results. This white color is due to the presence of air between the cells and can be made to disappear if the cankers are immersed in water. These excised cankers were then killed in strong alcohol, embedded in paraffin, sectioned, and stained with carbol fuchsin. This stain renders the bacteria bright red, making it easily possible to determine their position within the tissues.

Contrary to Hasse's observation, the bacteria teem around and between the host cells, being present in especially large numbers in the intercellular spaces (fig. 5). When the organism occurs within the

cells, one is led to conclude, since they appear to be confined to such cells, that entrance was effected after some mechanical rupture of the host cells.

A microscopic examination of sections of young spongy cankers in which there has been no desiccation from contact with the air shows that the host cells are not killed at first. Instead, they are considerably hypertrophied and become lightly attached to each other, as shown in figures 6 and 7. In fact, if fresh cankers are cut off with a sharp razor and mounted on a slide in a drop of water, some of the host cells separate intact and of their own accord from the mass of cankerous tissue. Little if any hyperplasia is believed to occur. It is highly improbable that cell division would occur in cells in which such profound changes were taking place. It is evident from figures 6 and 7 that the enlargement of cells already present would account for the production of the cankerous tissues. The same is believed to be true in Plate X, figure 1, illustrating Hasse's observations. It is not clear, however, from her explanatory statement that "there is a rapid development of cells" whether hypertrophy or hyperplasia is meant. Death of cells in the later stages of development of canker is probably caused by drying (Pl. IX, fig. 5). The dried cankerous tissues gradually become suberized.

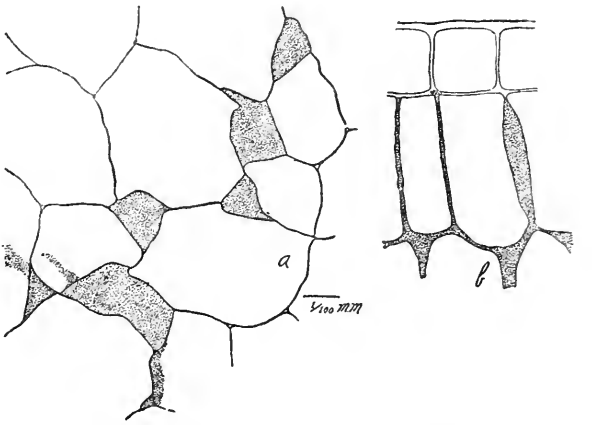


FIG. 5.—*Pseudomonas citri:* (*a*), In the mesophyll tissue and (*b*) in the palisade parenchyma. This material was fixed in strong alcohol, infiltrated with paraffin, sectioned, and stained with carbol fuchsin. Outlined with a camera lucida.

To explain the enlargement of the cells and their separation from each other, two hypotheses are advanced: First, the middle lamellæ are dissolved by an enzym, pectinase, secreted by the bacteria; second, osmotic pressure of the colloidal cell contents is modified so that the cells have a greater affinity for water. Evidence in support of both hypotheses has been secured which in part, at least, explains these interesting phenomena.

An attempt was made to demonstrate the secretion of pectinase by *Pseudomonas citri* by the following method: Six flasks of bouillon were inoculated with pure cultures of the organism. It was realized that the production of enzyms is largely dependent on the nature of the culture medium and that pectinase might be formed only within the host tissues. For this reason grapefruit leaves were placed in three of these flasks of bouillon prior to their sterilization and inoculation. After the organism

had grown in the flasks for four weeks, the bouillon was filtered through a Chamberland filter. This filtrate contained no living organisms, as demonstrated by transfers of platinum loopfuls to agar plates, with no growth on these plates after three days. When at the end of three days it was known that the filtrate was sterile, fresh grapefruit leaves were introduced into the filtrate. These leaves were sterilized, prior to their introduction, by immersion for half a minute in 1 to 1,000 bichlorid of mercury and by rinsing them subsequently in three changes of boiled tap water. Negative evidence of the presence of living organisms in the filtrate containing the grapefruit leaves was secured by agar plates made one week after the introduction of the leaves into the filtrate. An examination of the leaf tissues at the end of two weeks showed no evidence of dissolution of the middle lamellæ. This was true in the case of the filtrate obtained from both sets of the six original flasks.

In another experiment Irish potatoes were cut into slices and placed in moist chambers on moist filter paper. *Pseudomonas citri* was then

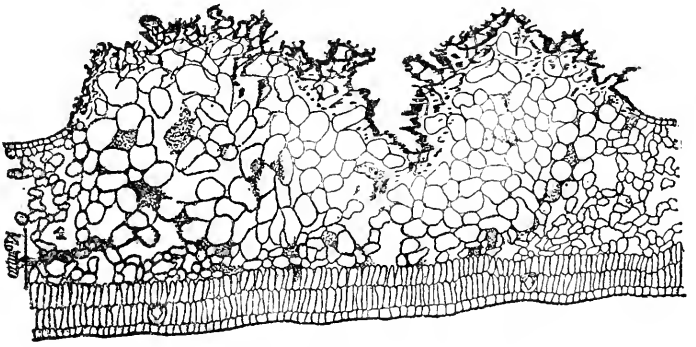


FIG. 6.—Drawing of a stained section of a natural canker on grapefruit.

transferred to these cut surfaces. Within a week hemispherical areas in which the cells were easily separable one from the other had been formed immediately beneath the colonies. That *P. citri* alone had caused this condition was shown by the reisolation in pure culture of this organism from the softened potato tissues. Because of this result, together with the fact, previously indicated, that the cells of cankerous tissues are so easily separable, and in spite of the negative evidence of enzym secretion in bouillon culture, it is believed that pectinase is secreted by the parasite.

The fact of the increased size of cells of cankerous tissue in itself supports the hypothesis that there has been an increased osmotic pressure within affected cells. Several facts contribute toward solving the question of how this increased pressure is brought about. In the first place the cell contents must manifestly be modified by the dissolution of the middle lamellæ, since there would be a tendency toward the establishment of equilibrium between the solution between the cells and the cell sap. Again, the growth of the organism between the cells with the consequent passage

of nutritive substances through the cell walls must exert an influence on the concentration of the cell sap. Then, too, the gelatinous material making up the bacterial cell walls certainly possesses considerable power of imbibition.

Further it has previously been pointed out that *Pseudomonas citri* exerts a strong diastatic activity when grown on potato cylinders. The production of this enzym has also been demonstrated by growth on starch agar prepared according to the method described by Crabill and Reed (4). Within a week a clear halo around the edge of the bacterial colony is formed on this substratum, thus making a striking ocular demonstration of dissolution of starch by the canker organism. If diastase, secreted by this organism, is readily diffusible through the cell walls, and it is reasonable to suppose that it is, it can convert the relatively insoluble starch into more soluble carbohydrates and thus increase the osmotic pressure of the cell sap.

It is not impossible that these several causes of increased osmotic pressure operating conjointly or separately may so profoundly modify the imbibitory properties of certain colloidal substances within the cells that their affinity for water is in consequence greatly increased.

No attempt has been made to determine the isotonic coefficient of the cell contents of the enlarged cells, but for the reasons just mentioned it is believed to be greater than that of normal cells.

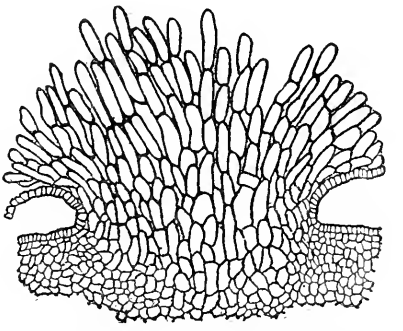


FIG. 7.—Cross section in outline of a spongy canker on the rind of a fruit of *Citrus decumana*, showing ruptured epidermis and hypertrophy of the rind tissues, the cells of which are loosely attached.

DISINTEGRATION OF THE TISSUES

An attempt has been made to gain certain information relative to the organisms involved in the disintegration of cankerous tissues, together with the nature of their activity on this tissue. It was previously pointed out that a species of Phoma is commonly associated with Citrus canker. Two other species of fungi belonging to the genera Gloeosporium and Fusarium are also sometimes present. Since certain bacteria and fungi are known to possess the power of hydrolyzing cellulose (13, 15), of which complex substance cell walls are largely constituted, an effort has been made to study the action of the organisms associated with canker upon pure cellulose. For this purpose cellulose agar was prepared according to the following method. Schweitzer's reagent was first made by adding ammonium chlorid and then an excess of sodium hydrate to a solution of copper sulphate. The blue precipitate thus formed was washed, pressed on a cloth filter, and dissolved in ammonium hydrate (sp. gr. 0.92). In this solvent 15 gm.

of sheet filter paper were dissolved, the solution was diluted about 10 times with water, and the cellulose was precipitated with a 15 per cent solution of hydrochloric acid. After considerable dilution the mixture was filtered, and the residue was washed repeatedly with water to remove all copper and chlorin. This residue was added to an agar medium consisting of agar, 10 gm.; monopotassium phosphate, 1 gm.; magnesium sulphate, 1 gm.; sodium chlorid, 1 gm.; ammonium sulphate, 1 gm.; calcium nitrate, 0.5 gm.; and the whole was made up to 1,000 c. c.

Poured plates of cellulose agar were made during May, inoculated with *Pseudomonas citri*, *Phoma* sp., *Gloeosporium* sp., and *Fusarium* sp., and incubated at room temperature. All grew poorly and none of the fungi fruited on this medium. There was no evidence of the production of cellulase except by *Phoma* sp. Within two weeks this organism had formed clear translucent halos as shown in Plate IX, figure 5, indicating that the cellulose had been hydrolyzed. Even though *Phoma* spp. strongly dissolve paper cellulose, they may not behave in this manner toward cell walls of *Citrus* spp., since other carbohydrates present would be more readily available than cellulose.

A further effort has been made to determine what other enzyms are secreted by these organisms and what part they might consequently play in the destruction of the tissues. Accordingly, Knop's mineral nutrient solution was prepared for use as a stock solution. This stock solution was then tubed and sterilized. To one set of these tubes of Knop's solution starch was added, to another saccharose, and to another maltose. They were then set aside and tested to determine whether they were sterile. It had previously been determined that sterilization subsequent to adding the carbohydrates resulted in a certain amount of conversion of these carbohydrates. When it was determined that they were sterile, four sets of four tubes each were taken of each of the nutrient solutions. Three tubes in each set of four were inoculated with pure cultures of one of the four organisms mentioned above and one tube in each set was left as a check. After 10 days the solutions were tested, with the following results: Fehling's solution showed a strong reduction in the starch solutions in which *Pseudomonas citri* and *Phoma* sp. had been grown, showing the production of diastase. There was no change in the checks nor in the solutions in which the other organisms were grown.

Inversion of saccharose, as evidenced on the reduction of Fehling's solution, had been accomplished in the solutions in which *Phoma* sp. and *Fusarium* sp. had been grown, indicating the presence of invertase. Positive tests for dextrose or glucose were secured with Barfoed's reagent and with Nylander's reagent in these inverted saccharose solutions. Negative results were secured with the other organisms and with the checks.

Phoma sp. alone seemed to have any action on maltose. Inversion into dextrose was shown by positive tests with Barfoed's reagent.

Negative tests for lipase production were secured in the case of each of the four organisms.

From the foregoing tests it is seen that *Phoma* sp. secretes cellulase, diastase, invertase, and maltase, and must therefore be regarded as very destructive to the carbohydrate material of diseased tissues. Cellulase very probably aids in the destruction of the cell walls; diastase converts the starch into maltose and dextrin and then further acts on the dextrin. When a few drops of iodin were added to a starch solution in which *Phoma* sp. had grown, blue and red colors developed, indicating amylo- and erythro-dextrin. Maltase probably further reduces the maltose to dextrose.

It has also been found that *Phoma* sp. affects the acidity of the medium upon which it is grown. This was determined by growth in pure culture of the fungus on leaves and fruits of *Citrus trifoliata*. This material was first macerated by passing it through a meat chopper. Thirty-gm. samples of ground leaves and of fruits were then placed in 250 c. c. Erlenmeyer flasks and were sterilized in an autoclave. After sterilization some were inoculated with *Phoma* sp. from pure cultures and others left as checks. A copious white growth occurred on those which had been inoculated. After a month 150 c. c. of distilled water were added to each of the flask cultures and to the checks. The flasks were then heated on a water bath for 30 minutes, the liquid filtered through asbestos, and 25 c. c. of the filtrate taken for titration, using *N/20* sodium hydroxid, with litmus paper as an indicator. The following is representative of the results obtained: 7.1 c. c. of *N/20* sodium hydroxid neutralized 25 c. c. of the filtrate from the leaves in the check flask and 9.8 c. c. that from the fruits in the check flask. The filtrate from the leaves upon which *Phoma* sp. had been growing was neutral to litmus, and that from the fruits required 1.3 c. c. of *N/20* sodium hydroxid to neutralize it. From this it is concluded that *Phoma* sp. is able to utilize the organic acids as a source of food, a condition contrary to that which Hawkins (7) found in a study of the chemical changes produced by the brown-rot fungus on peaches.

TAXONOMY OF THE FUNGUS

An effort has been made definitely to assign this species of Phoma to one of the numerous species of the form genera Phoma and Phyllosticta, which have previously been described as occurring on parts of *Citrus* spp. The pycnidia of the species under consideration are globose, ostiolate, 100 to 150μ in diameter (Pl. XI, fig. 5, 6) and wholly or partially embedded within the cankerous tissue. The pycnidial walls are thin, being thickest around the ostiolum, and are very similar in color to the corky brown host cells. The conidia are elliptical or oblong in outline,

hyalin, and 9 to 12 by 3 to 4μ. They germinate within 24 hours in water or in a variety of culture media. A white mycelial growth is produced on bean agar. Pycnidia are readily formed on agar (Pl. XI, fig. 3) modified by the addition of a water extract from corn meal, rice, cowpeas, orange stems, etc. (fig. 8).

The fungus, furthermore, was very probably introduced into the United States similtaneously with *Pseudomonas citri*. It is impossible to determine the position of this organism among previously described species, since it has been found to be morphologically not unlike several of them. Its relation to the production of Citrus canker is definitely established as a result of this study. Then, too, no particular difficulty would be experienced by other investigators in identifying it because of its association with Citrus canker. In view of these facts it seems well to describe it as a new species with the following brief technical diagnosis:[1]

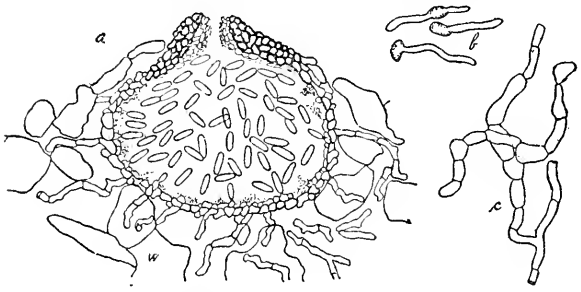


FIG. 8.—*a*, Cross section of a pycnidium of *Phoma socia* from a grapefruit leaf. This material was fixed in chromo-acetic acid, embedded in paraffin, sectioned, and stained in saffranin and gentian violet. Drawing outlined with the aid of a camera lucida. *b*, Germination of conidia of *Phoma socia* after 24 hours in water. *c*, Mycelium of this fungus in old cultures.

Phoma socia, n. sp.

Pycnidia irregularly distributed, globose, wholly or partially embedded, 100 to 150μ in diameter; walls thin, corky brown in color, thickened only around the ostiolum, which opens centrally; conidia continuous, elliptical or oblong, hyalin, 9 to 12 by 3 to 4μ.

Occurs in the cankers produced by *Pseudomonas citri* on living leaves and branches of *Citrus trifoliata*, *C. nobilis*, and *Fortunella* sp. and on living leaves, branches, and fruits of *C. decumana* and *C. aurantium*.

ACIDITY AND RESISTANCE TO CANKER

It is generally conceded by both nurserymen and growers and has been substantiated by the field observations of the writer that Satsuma oranges are not as susceptible to Citrus canker as grapefruit. This difference may be noted when both species are grown in locations where they are equally exposed to infection. The tolerance of bacteria to acidity has been found to be relatively low. Resistance to certain fungus diseases, as, for example, the resistance of hard wheat to rust, has been found (3)

[1] **Phoma socia**, sp. nov.

Peritheciis irregulariter distributis, globosis plus minusve immersis, 100 to 150μ diam.; contextu membranaceo, corticale-brunneo, cum cellulis circa ostiolum pseudoparenchymaticis, centro perforatis; sporulis continuis, ellipticis v. oblongis, hyalinis 9–12 × 3–4μ. Hab. in foliis ramisque, vivis *Citri trifoliatae*, *C. nobilis* et *Fortunellae* sp. et quoque in foliis, ramis fructibusque *C. decumanae* et *C. aurantii*. Socia adest *Pseudomonas citri* Hasse.

to be correlated with the acidity of the cell sap. Because of these several facts, an effort has been made to determine whether the difference in susceptibility between Satsuma oranges and grapefruit can be accounted for on the basis of difference in acidity. Leaves collected from plants growing in the greenhouse were used in these tests. The leaves were finely macerated by trituration; distilled water was then added to make a volume equaling 200 times the weight of the finely ground leaves; phenolphthalein was added as an indicator; and the acids present in the sample were titrated with *N/10* sodium hydroxid. This method is open to criticism where absolutely accurate determinations are sought, but is regarded as satisfactory in indicating relative differences. Considerable variations in acidity of the same species were noted, dependent largely upon the cessation of photosynthetic activity at night. Greater acidity, as would be expected, occurred in samples collected early in the morning. Representative results of these tests, however, are shown in Table I.

TABLE I.—*Acidity of oranges and grapefruit*

Variety.	Wet weight of tissue.	Quantity of *N/10* sodium hydroxid to neutralize 1 gm. wet weight of tissue.		Percentage of moisture in sample.	Percentage of total acidity based upon average water content.
		Actual.	Average.		
	Gm.	*C. c.*	*C. c.*		
Satsuma (old).....	3.62	1.0359	1.0184	60.2	1.691
	3.06	.9967			
	4.14	1.0406			
	4.17	1.0431			
	2.92	.9760			
Satsuma (young)..	2.60	.9423	.9735	67.8	1.465
	2.90	1.0172			
	2.55	.9609			
Grapefruit (old)...	2.71	.8672	.8787	59.0	1.490
	2.53	.8695			
	1.66	.8434			
	2.11	.9005			
	1.95	.9128			
Grapefruit (young).	1.97	.8629	.8634	79.9	1.080
	2.03	.8620			
	1.56	.8653			

The leaves of Satsuma oranges are consistently higher in acid content than those of grapefruit, since the former require 1.0184 c. c. of *N/10* sodium hydroxid to neutralize 1 gm. of wet weight of leaf tissue, young Satsuma leaves, 0.9735 c. c., old grapefruit leaves, 0.8787 c. c., and young grapefruit leaves, 0.8634 c. c. When the acidity of the cell sap is computed on the basis of the total moisture content of the leaves, it is found to be 1.691 per cent for old Satsuma leaves, 1.465 per cent in those of young Satsumas, 1.490 per cent in those of old grapefruit, and 1.080

per cent in those of young grapefruit. It will be recalled that bacterial growth occurs on artificial media rendered acid by hydrochloric or citric acid when a sufficient amount of acid has been used to make the acidity of the media 2 per cent. The acidity of the leaf tissue is therefore not sufficient to inhibit the growth of the canker organism and is not regarded as sufficient to account for the difference in susceptibility. No determinations have been made of the kinds and relative amounts of the several organic acids in the tissues of the two species. Until this is known there still remains the possibility of a correlation between susceptibility to canker and acidity.

CHEMICAL CHANGES IN CITRUS LEAVES BROUGHT ABOUT BY CITRUS CANKER

Little attention has been given by the biochemist to the chemical transformations occurring in diseased plant tissues. Such studies would no doubt throw a flood of light upon the intimate relationship of parasite and host and would materially contribute to our knowledge of the nature of parasitism. The literature dealing with the chemical changes induced by plant pathogens is more or less fragmentary, mainly because of the inexact state of our knowledge regarding the separation and quantitative estimation of the various compounds occurring in plant tissues. An historical résumé of this literature has therefore been purposely omitted. However, among the recent excellent papers along this line may be mentioned the work of Hawkins (7) upon the changes in peaches induced by the brown-rot organism, *Sclerotinia cinerea*. He found in brown-rotted tissues an increase in acid content, a decrease in certain alcohol-soluble substances, a decrease in the total sugar content, and practically a disappearance of the cane sugar. It was with the view of determining something of the changes produced by Citrus canker that this portion of the investigation was undertaken.

Diseased and healthy leaves were taken from grapefruit trees affected with Citrus canker. Circles of diseased tissue and tissue from healthy leaves were excised with a cork borer. These leaf circles were then triturated in a mortar until the material was finely divided, their wet weight determined, 27.25 gm. in each case, and preserved in such volume of 95 per cent alcohol that the alcohol concentration of the mixture was 85 per cent. This concentration could not be accurately made until it had been determined that the moisture content of normal leaves was 61.69 per cent and that of diseased leaves 61.57 per cent. The material was then set aside for two weeks and was shaken occasionally to permit the gradual extraction of the cold alcohol-soluble portions. The method followed subsequently was based upon those devised by Koch (9, 10, 11, 12) for use in the quantitative chemical analysis of animal tissues.

This method consists essentially in the separation of the material into three fractions. Fractions 1 and 2 consist of the soluble portion extracted by the action of alcohol, ether, and water, and fraction 3 consists of the insoluble residue. Fractions 1 and 2 are separated by lipoid precipitation. The former fraction contains precipitated lipoids, while the latter contains all nonlipoid materials, soluble in alcohol, ether, and water. Instead of the modified Wiley extraction apparatus employed by Koch and his pupils, a rubber analysis extraction apparatus (8) has been employed. Extractions in this apparatus, like those with the modified Wiley apparatus, are carried out at the boiling point of the solvent.

In making the first alcohol extraction the preserved material was transferred to Schleicher and Schüll extraction thimbles, previously fitted into the siphon cups of the extraction apparatus. The preserving liquid was then filtered through these thimbles. Perforated porcelain plates or filter paper cut to fit were then used as covers over the material in the thimbles.

Extraction for 12 hours with redistilled 95 per cent alcohol followed. The alcohol was changed two or three times during this extraction in order to prevent possible decomposition of extracted materials in the boiling alcohol. The tissue was pressed to remove the excess alcohol, and an ether extraction was made. This extraction was continued for 12 hours for the purpose of facilitating the subsequent powdering of the tissues. The material was then removed from the extraction thimbles to a mortar and was ground to a powder. This powder was placed in a stoppered flask with a volume of distilled water equaling twice the fresh weight of the material and was boiled on a steam bath for two hours. Warm absolute alcohol was added in a sufficient quantity to bring the alcohol content of the whole up to 90 per cent. The mixture was warmed on the bath, with repeated shaking, and set aside until the following day. It was then filtered through the original extraction thimbles and extracted for 12 hours with 95 per cent alcohol. At the close of this extraction the residue in the cups (fraction 3) was transferred to previously weighed porcelain crucibles and dried to constant weight in an oven at 100° C. By this procedure the alcohol, ether, and water-soluble portions (fractions 1 and 2) were separated from the insoluble portion (fraction 3).

In further preparing the soluble portions of the material for analyses they were combined only after the ether-soluble portion had been heated on a water bath until the odor of ether could no longer be detected. A little alcohol was added from time to time to take up the materials left behind by the loss of ether by evaporation. In pouring the solutions together a precipitate appeared which was rendered soluble by the addition of sufficient hot water to bring the alcohol concentration down to 70 per cent. The solution was then made up to 2,000 c. c., 200 c. c. of which were taken for the estimation of solids. The remainder was

evaporated at 75° C. to a sirupy consistency or until all the alcohol had evaporated and the sirupy mass was emulsified with warm water. This emulsion was placed in a stoppered volumetric flask, shaken with 20 c. c. of chloroform, 10 c. c. of hydrochloric acid were slowly added, and then it was made up to a given volume by the addition of water. The flask was then placed for 48 hours in running water under the hydrant to facilitate the precipitation of the lipoids in the chloroform. Filtration followed, the filtrate constituting fraction 2, and the lipoid precipitate on the filter paper fraction 1. The precipitate was then taken up with a large volume of hot, 95 per cent alcohol, and kept on a water bath at 75 C., until all of the chloroform was driven off. The volume was then increased to a convenient amount, and aliquot parts taken for analyses.

The analysis of fraction 3 included (*a*) total phosphorus, (*b*) total nitrogen, (*c*) cellulose, (*d*) carbohydrate after hydrolysis, (*e*) ash, (*f*) total solids; the analysis of fraction 2 included (*a*) dry weight and ash made upon an aliquot part, (*b*) total sugars before and after hydrolysis, (*c*) total nitrogen, (*d*) phosphorus, (*e*) solids; while the analysis of fraction 1 included only (*a*) total solids, (*b*) phosphorus, (*c*) nitrogen, since the total weight of the lipoidal material from the two samples differed by 1 mgm. only and since the amounts were too small to admit of accurate separation.

The determinations of phosphorus were made upon aliquot parts by the Pemberton-Neuman method described by Mathews (14, p. 893–895).

The total nitrogen was determined upon all fractions by the employment of the Gunning-Arnold modification of the Kjeldahl method. No determinations were made of fatty acids.

In fractions 2 and 3 the carbohydrate determination included reducing sugars, total sugars, and cellulose. Prior to the determination of reducing sugars, the solution was freed from organic acids, tannins, and other substances capable of affecting reduction by Fehling's solution. This was accomplished by treatment with lead subacetate in excess, after which the solution was diluted, filtered, and saturated sodium sulphate was added to precipitate the excess of lead. The clear filtrate was then diluted, and an aliquot part taken for the determination of reducing sugar by the Bertrand volumetric method. The reducing sugar was calculated as dextrose by the Munson and Walker tables.[1] Another aliquot part of the solution, a part of which had been used for the determination of reducing sugars, was used upon which to determine the total sugars. This was hydrolyzed by the addition of concentrated hydrochloric acid, following which the solution was kept on a water bath at 69° to 70° C. for 10 minutes. It was then cooled, neutralized with 40 per cent sodium hydroxid, and the sugar determined as invert sugar by the volumetric permanganate method.

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 241–251. 1908.

Cellulose determinations in fraction 3 were made in duplicate with accordant results by employing Schweitzer's reagent in one case and a solution of zinc chlorid in hydrochloric acid in the other.

Polysaccharids in fraction 3 were estimated as dextrose after 2.5 hours' hydrolysis in a reflux condenser using 2.5 per cent hydrochloric acid.

In Table II are given the fresh weights of normal and cankerous Citrus tissue, moisture content, dry weight, and alcohol-ether soluble and insoluble portions.

TABLE II.—*Analysis of normal and cankerous tissue of grapefruit leaves*

Item.	Normal tissue.	Cankerous tissue.
	Gm.	*Gm.*
Fresh weight	27.250	27.250
Moisture	16.799	16.796
Dry weight	10.451	10.454
Total alcohol-ether:		
Soluble	4.270	4.300
Insoluble	6.181	6.154

In this composite table it is strikingly significant that only slight differences are apparent. The moisture content of normal tissue is slightly greater than that of cankerous tissue, and there is, of course, a corresponding decrease in dry weight. The greater amount of alcohol-ether soluble material occurs in cankerous tissues with a lesser amount of alcohol-ether insoluble substance. The differences represented herein would have little or no value in themselves if it were not that they were obtained by the use of a refined method of analysis primarily intended to permit the discovery of changes not indicated by ordinary methods. Studies of the intricate relation of parasite and host have proceeded far enough to indicate that large changes in composition of the host are not to be expected, but rather that transformations have been produced which, though minute in amount, profoundly affect the metabolism of both parasite and host. Table III gives in detail the results of the several steps in this analytical procedure.

TABLE III.—*Analyses (in grams) of normal and cankerous grapefruit leaves*

Item.	Fraction 1.		Fraction 2.		Fraction 3.		Totals.	
	Normal.	Diseased.	Normal.	Diseased.	Normal.	Diseased.	Normal.	Diseased.
Dry weight	0.781	0.780	3.479	3.520	6.181	6.154	10.441	10.454
Nitrogen	.0196	.686	.1190	.1120	.105	.0818	.2436	.2624
Phosphorus	.0123	.0114	.021	.0225	.0101	.0131	.0434	.0470
Reducing sugars	.....	.....	2.008	.806	.....	.....	2.008	.806
Sugar after acid hydrolysis	.....	.....	.387	.284	.573	.370	.960	.654
Polysaccharids (soluble)	.....	.....	.....	.....	.2087	.1273	.2087	.1273
Cellulose	.....	.....	.....	.....	.859	.843	.859	.843
Ash	.....	.....	.....	.....	.041	.041	.041	.041

It should be stated with reference to the data presented in Table III that the figures given are the weights in grams of the several constituents as determined by employing 27.25 gm., fresh weight, of healthy and of cankerous tissue. Since the two samples differed by only 3 mgm. in dry weight and since the figures, to be directly comparable, should be based on dry weights in each case, a correction of 0.24 per cent should be applied to the analyses of diseased tissue. As this is insignificant, the data are regarded as referable, and the corrections have not been applied.

Because of the presence of certain enzyms, of which mention has been made earlier in this paper, it is to be expected that the changes of greatest magnitude would occur in the carbohydrates. That such is the case is obvious when one notes in the totals given in Table III a reduction of all classes of carbohydrate in cankerous tissue. Thus, in equal quantities of fresh material the amounts of reducing sugar are found to be as 5 to 2, the total sugars as 3 to 2, and the polysaccharids as 5 to 3 when normal and diseased tissues are compared. Because of the ease with which they are available to the invading organisms, the reducing sugars are probably the most strongly attacked. After acid hydrolysis the normal tissue shows more reducing sugar than the diseased, both in the alcohol-ether soluble and alcohol-ether insoluble fractions. This means that there is also a less amount of the higher soluble carbohydrates, disaccharids, in diseased tissues and that they too are more easily available than the polysaccharids. The ratio of disaccharids in normal and cankerous tissue in the alcohol-ether soluble and alcohol-ether insoluble portions is as 3 to 2 and 5 to 3, respectively.

There is also a slight but significant decrease in the amount of cellulose found in diseased tissues. Although the difference in total cellulose in the normal and diseased tissues is slight, the results given are representative of a considerable number of determinations in which two standard methods were employed and in which the lesser amount of cellulose was invariably found in the diseased tissue. Experimental error has thus been eliminated and the results indicate a slight but unmistakable destruction of cellulose by the invading organisms.

The polysaccharids were determined in fraction 3 after 2.5 hours acid hydrolysis. They were found to be present in normal tissues and diseased tissues in the same proportion, 5 to 3, as were the disaccharids. There has therefore been a corresponding reduction and utilization of both di- and poly-saccharids by the invading organisms.

In the alcohol-ether insoluble fraction the amounts of nitrogen found for normal and diseased tissue were 0.105 and 0.0818 gm., respectively. If the conventional factor for these figures, 6.25, is employed, 0.654 and 0.511 gm. are obtained as the protein content of normal and diseased tissues, respectively. The protein content of diseased tissue has therefore been reduced 78.16 per cent. One should therefore expect to find a

very material increase in the nitrogen of the alcohol-ether soluble portion of the diseased tissue. This expectation is realized, since the nitrogen figures for the soluble portions are 0.1386 gm. for normal and 0.7806 gm. for diseased tissue. This represents an increase in the diseased tissue of 37.52 per cent over the healthy tissue. This increase in the soluble portion indicates a decomposition of the complex nitrogenous compounds resulting in the formation of peptones and amino acids soluble in alcohol and ether. This difference in nitrogen content of the alcohol-ether soluble portion takes an added significance when the nitrogen content of fraction 1 and that of fraction 2 are examined separately. It will be recalled that the nitrogen of fraction 2 represents those portions of the nitrogenous constituents extracted by alcohol and ether which are readily soluble in water after the combined extract has been evaporated to a paste. They are, therefore, amino acids and polypeptids. It will further be recalled that fraction 1 is obtained from the watery solution of the alcohol-ether soluble extract by chloroform precipitation and is therefore lipoid nitrogen. The slight decrease in nitrogen in fraction 2, when normal and diseased tissue are compared, is accompanied by an enormous increase, amounting to 250 per cent in the lipoid nitrogen of fraction 1.

These differences in nitrogen content of the several fractions lend themselves to two possible explanations. The first and most obvious interpretation of the results is that the changes produced by the invading organisms in the proteins of the host result in the formation not of amino acids and other end products of protein decomposition but in the production of complex intermediate substances. The other explanation is based upon the fact that the bacteria themselves derive the nitrogen necessary for the building of their own proteins as well as for the formation of their cell walls from the proteins of the host. Concurrently with the reduction of the protein of the host to simpler forms a series of metabolic processes is occurring within the invading organism which involves the synthesis of these simple nitrogenous compounds to more complex ones. The changes in nitrogen content of the several fractions of the diseased tissue are therefore the result of both analytic and synthetic processes. At present it is impossible to employ any methods, as none have been devised, which will indicate what the end products of decomposition of the host proteins by the invading organism are, since the formation of these products is accompanied by their concomitant utilization in the manufacture of new compounds peculiar to the body of the parasite.

The total phosphorus in the diseased tissues is greater in amount in fractions 2 and 3 than in the normal tissues. Were the changes in the diseased tissue purely katabolic, it would be expected that there would be a material increase in water-soluble phosphorus derived from the

decomposition of neucleoproteins. On the contrary, the phosphorus of fraction 3 shows an increase of 30 per cent, that of fraction 2 an increase of 20 per cent, and that of fraction 1 a decrease of about 7 per cent. The increase in water-soluble phosphorus in fraction 2 indicates that decomposition processes are taking place, but the concomitant increase in phosphorus content in fraction 3 shows that such decomposition is accompanied by actual synthetic processes involving the use of phosphorus.

No difference appears between the two tissues in amounts of ash as shown in fraction 3. The ashing of fractions 1 and 2 gave unsatisfactory results and for this reason the figures are withheld.

It is evident from the foregoing statement of results that the significant changes brought about in diseased tissues concern carbohydrate and nitrogenous constituents. The concurrent disappearance of mono-, di-, and poly-saccharids from diseased tissues indicates that all the sucroclastic enzyms previously shown to be formed by the organisms in pure cultures are active in the host tissues and that the reducing sugars formed are utilized by the organisms as sources of energy. The results with nitrogen indicate that there is not an accumulation of the products of protein decomposition but that the destructive transformation of protein is accompanied *pari passu* by a utilization of the decomposition products in the anabolic processes of the organisms.

AGENCIES CONCERNED IN DISSEMINATION OF CITRUS CANKER

Definite experimental data are wanting on the agencies by which Citrus canker is spread. If we judge, however, from field observations and from a knowledge of other bacterial plant diseases, it is evident that rain and dew are important factors in carrying the disease to unaffected leaves, twigs, and fruits of trees in which the diesase is already present. Man himself is a very important agent in effecting the distribution of canker from diseased trees to healthy trees near by. When in the cultural operations of budding, cultivation, picking, etc., he comes in contact with diseased trees and soon afterwards touches healthy ones, infection may result. The chances of infection are greatly increased if he comes in contact with newly formed cankers on the diseased trees, and if a film of moisture is present on the adjacent healthy trees which he may touch. The most plausible explanation of the introduction of Citrus canker into two groves which have come under the writer's observation is through the agency of man. The owners had visited groves in which canker occurred in order to acquaint themselves with the appearance of the disease. On returning home they examined certain of the trees in their own groves and these trees soon afterward developed canker lesions. Stirling (2) reports the transmission of the disease through handling diseased leaves prior to touching healthy ones. It is highly probable that

certain birds and insects also effect this contact of diseased with healthy parts and are therefore to be regarded as agents in dissemination of Citrus canker.

CONTROL OF THE DISEASE

During the summer of 1914 those who had been attempting to solve the problem of controlling Citrus canker realized that it was an exceedingly difficult undertaking. Efforts were directed along three lines: Exclusion, protection, and eradication.

EXCLUSION.—Those interested in the welfare of the Citrus industry in Florida were the first to realize the serious nature of Citrus canker and that it had been introduced into the State from other States and from foreign countries. For these reasons a quarantine was imposed during the spring of 1914 to prevent the further introduction into Florida of Citrus trees and buds and thus of Citrus canker. Other of the Gulf States later in the season realized the jeopardy in which their Citrus growers' interests were placed and issued similar regulatory measures on the importation of shipments of Citrus stock. On January 1, 1915, a Federal quarantine was imposed to exclude the further importation of this disease into the United States. The agitation throughout the entire Citrus growing section of the Gulf coast attendant on the adoption of these regulations looking toward control by exclusion have so familiarized the growers with Citrus canker that it is unnecessary to advise the exercise of care in ordering trees to be used in setting out a Citrus grove. It is realized that in no case is it safe to purchase trees from nurseries in which this disease occurs.

PROTECTION.—Since certain fungicides have been successfully used in the control of various Citrus diseases a number of experiments were undertaken during the spring of 1914 to determine the effectiveness of these mixtures in the control of Citrus canker. A grove of badly diseased grapefruit was used upon which to make applications of Bordeaux mixture, ammoniacal copper carbonate, and soluble sulphur. Details of these experiments are withheld, since it was realized early in the summer that the application of these fungicides was without appreciable effect in the control of canker.

Again in the spring of 1915 another grove of grapefruit was selected in which to test the effectiveness of several fungicides in protecting the trees from infection by the canker organism. All visible signs of canker were carefully removed from the trees prior to the application of the mixtures. Bordeaux mixture, Bordeaux mixture and bichlorid of mercury (12 tablets in 3 gallons), Bordeaux mixture and formaldehyde (1 : 100), and a Bordeaux and lead arsenate mixture were employed. Applications were made on March 26, April 29, and May 14, and no new infections had developed on any of the sprayed or unsprayed trees by the last-named date. On May 27, however, new infections were apparent

and were equally numerous on sprayed and check trees. A number of growers have used various germicidal mixtures in attempts to find a preparation which could be successfully employed against Citrus canker. In no case have these efforts met with a sufficient degree of success so that their use in canker control can be recommended. When formaldehyde is used in sufficient strength to cause the death of the leaf tissues in a considerable area surrounding the cankers no viable organisms can be found in the cankerous tissues in many cases. They are still viable, however, in others, and it has also been found to be impossible to cause formaldehyde to penetrate sufficiently deep into old suberized limb cankers to kill the canker organisms. In the light of these tests and in the light of the ineffectiveness of sprays in the control of other plant diseases of bacterial origin, it is believed that there is little to be hoped for in the use of germicides for protection against Citrus canker.

ERADICATION.—The history of the work of eradication of Citrus canker, little of which has been published outside of the daily press, would in itself be voluminous, and it is not the present purpose to include it in this account. The early efforts toward the eradication of Citrus canker were confined to the removal of diseased parts in case the trees were only slightly diseased. When the trees were seriously affected, however, they were severely pruned, even though this necessitated the removal of nearly all of the branches. Pruned trees were then thoroughly sprayed with Bordeaux mixture. It was recommended that all the diseased parts which had been removed should be burned.

After a few months' trial it was seen that by this procedure the treated trees were still diseased. Further than this, adjacent trees had become diseased, although they were apparently healthy at the time efforts had been made to remove cankered leaves and branches from the trees near by.

Even when the work of removal had been done by skilled hands and when the trees had received several applications of some fungicide to protect the new growth they were still found to become cankered.

As a result of this it was decided during the summer of 1914 that only the complete destruction of the diseased trees by burning would be effective. As a result of this decision the eradication campaign was organized and a concerted, heroic effort is being put forth to stamp out Citrus canker from the Gulf States. The intelligent observance of the strictest sanitary precautions with reference to trees adjacent to those which are destroyed is necessary.

SUMMARY

A serious disease, commonly known as Citrus canker, which affects species of Citrus and Fortunella, has within the past few years been introduced into Alabama and other of the Gulf States. It attacks fruits, leaves, twigs, and larger branches, producing characteristic cankerous

lesions. The primary cause of the disease is *Pseudomonas citri*, first isolated by Hasse from grapefruit and found to be pathogenic on grapefruit seedlings. This has been confirmed, and in addition an organism presenting the same cultural and physiological characters has been isolated from trifoliate and Satsuma oranges and lemons. No difficulty has been experienced in cross-inoculating the organism on McCarty and seedling grapefruit, Pineapple oranges, Satsuma oranges, and seedling trifoliate oranges. It grows readily on a variety of artificial media, and according to the studies made its group number is 221.3332513.

Infection occurs through natural openings and through wounds. The rapid spread of the disease is favored by the simultaneous occurrence of newly exposed cankerous cells and the presence of a film of moisture, especially on young parts of the plant. The bacteria occur for the most part between the cells of the host and cause them to become considerably hypertrophied. Little, if any, hyperplasia is believed to occur. This enlargement of the cells is caused by the dissolution of the middle lamellæ through enzym activity and by a modification of the host protoplast so that its osmotic pressure is increased. This increased pressure results from the presence of the parasite between the cells and from the passage of materials through the walls of the host, occasioned by the growth of the organism.

Besides *Pseudomonas citri*, fungi belonging to the genera Phoma, Fusarium, and Gloeosporium have been isolated from Citrus cankers. Of the fungi *Phoma* sp. alone was found to be notably active in the disintegration of the tissues. It is able by virtue of the secretion of specific enzyms to utilize the carbohydrates, cellulose, starch, maltose, and saccharose and causes also a decrease in acidity of invaded tissues. It is regarded as heretofore undescribed and is herein given the name "*Phoma socia*, n. sp."

The difference in susceptibility to Citrus canker of Satsuma oranges and grapefruit can not be accounted for on the basis of differences in total organic acids in the two hosts.

Comparative analyses of grapefruit leaves affected with Citrus canker and of healthy leaves shows that there has been in diseased leaves a decrease in all of the soluble and insoluble carbohydrates due to their utilization by means of sucroclastic enzyms secreted by the canker organisms. Apparently a decomposition of the host proteins occurs concurrently with their synthesis in the metabolism of the parasite proteins, and there results a slight increase in total nitrogen in diseased tissues. The slight increase in phosphorus in diseased tissues is accounted for in the same manner as that in nitrogen, since they appear to be correlated. No differences in ash were found in fraction 3, and the dry weight of diseased tissues was slightly greater than that of normal.

Rain and dew are important agencies in the dissemination of Citrus canker. Any other agencies, of which man is probably the most im-

portant, which effect a contact of diseased parts with healthy parts, are to be recognized as carriers.

In efforts to control the disease quarantine measures have been passed, thus preventing its further introduction from foreign localities and from any one of the Gulf States to any other of them. The use of spray mixtures indicates that they are not to be regarded as remedial measures of appreciable value in canker control; nor will their use protect healthy trees adjacent to diseased ones from infection.

Successful eradication seems possible, but only when the work of destruction of diseased trees is thoroughly done, with the observance of proper sanitary precautions.

LITERATURE CITED

(1) BERGER, E. W.
1914. Citrus canker—History. *In* Fla. Grower, v. 11, no. 7, p. 14–15.

(2) ——— STEVENS, H. E., and STIRLING, Frank.
1914. Citrus canker—II. Fla. Agr. Exp. Sta. Bul. 124, p. 27–53, fig. 7–14.

(3) COMES, Orazio.
1913. Della resistenza dei frumenti alle ruggini. Stato attuale della quistione e provvedimenti. *In* Atti R. Ist. Incorag. Napoli, s. 6, v. 64, 1912, p. 421–441. Letteratura e note, p. 437–440. *Abstract in* Internat. Inst. Agr. [Rome], Mo. Bul. Agr. Intel. and Plant Diseases, year 4 no. 7, p. 117–119.

(4) CRABILL, C. H., and REED, H. S.
1915. Convenient methods for demonstrating the biochemical activity of microorganisms, with special reference to the production and activity of enzymes. *In* Biochem. Bul., v. 4, no. 13, p. 30–44, pl. 1.

(5) EDGERTON, C. W.
1914. Citrus canker. La. Agr. Exp. Sta. Bul. 150, 10 p.

(6) HASSE, Clara H.
1915. Pseudomonas citri, the cause of citrus canker. A preliminary report. *In* Jour. Agr. Research, v. 4, no. 1, p. 97–100, pl. 9–10.

(7) HAWKINS, L. A.
1915. Some effects of the brown-rot fungus upon the composition of the peach. *In* Amer. Jour. Bot., v. 2, no. 2, p. 71–81. Literature cited, p. 80–81.

(8) JOINT RUBBER INSULATION COMMITTEE.
1914. Tentative specifications and analytical procedure for 30% Hevea rubber insulating compound. Preliminary report of the joint rubber insulation committee appointed by a group of manufacturers and users of rubber compounds, 1911–1914. *In* Jour. Indus. and Engin. Chem., v. 6, no. 1, p. 75–82, illus.

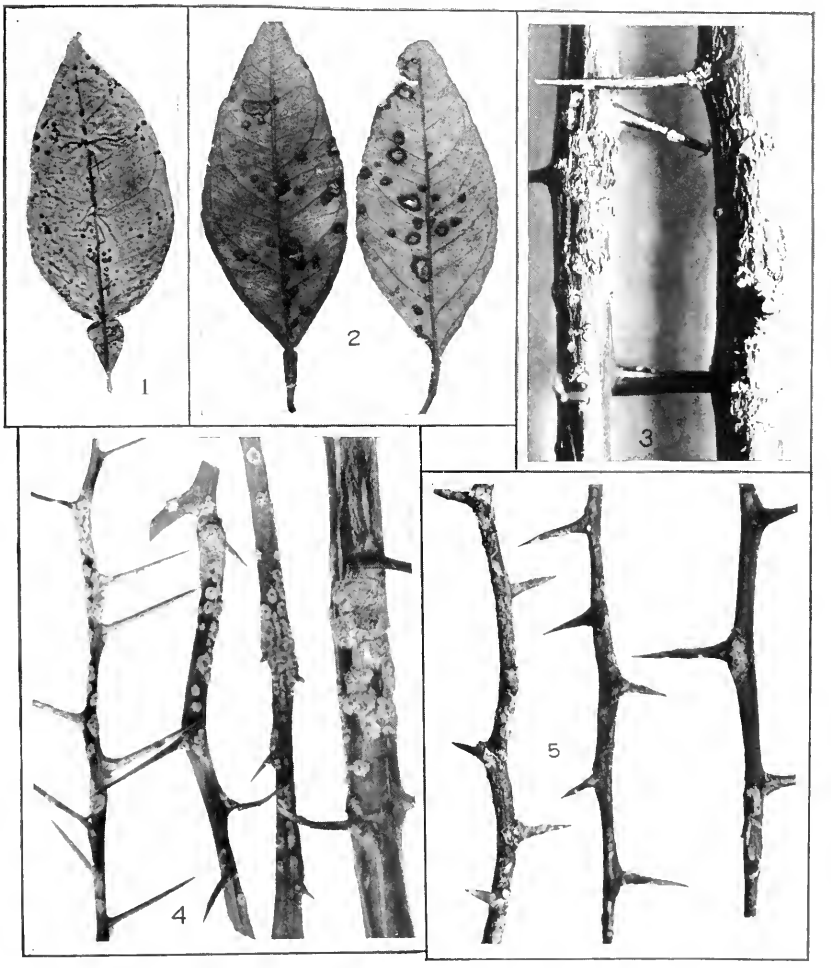
(9) KOCH, W.
1909. Methods for the quantitative chemical analysis of animal tissues. I. General principles. *In* Jour. Amer. Chem. Soc., v. 31, no. 12, p. 1329–1335.

(10) ——— and MANN, S. A.
1909. Methods for the quantitative chemical analysis of animal tissues. II. Collection and preservation of material. *In* Jour. Amer. Chem. Soc., v. 31, no. 12, p. 1335–1341.

(11) Koch, W., and Carr, E. P.
1909. Methods for the quantitative chemical analysis of animal tissues. III. Estimation of the proximate constituents. *In* Jour. Amer. Chem. Soc., v. 31, no. 12, p. 1341–1355, 1 fig.
(12) ——— and Upson, F. W.
1909. Methods for the quantitative chemical analysis of animal tissues. IV. Estimation of the elements, with special reference to sulphur. *In* Jour. Amer. Chem. Soc., v. 31, no. 12, p. 1355–1364, 1 fig.
(13) McBeth, I. G., and Scales, F. M.
1913. The destruction of cellulose by bacteria and filamentous fungi. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266, 52 p., 4 pl.
(14) Mathews, A. P.
1915. Physiological Chemistry * * * 1040 p., 78 fig. New York.
(15) Scales, F. M.
1915. Some filamentous fungi tested for cellulose destroying power. *In* Bot. Gaz., v. 60, no. 2, p. 149–153.
(16) Stevens, H. E.
1914. Citrus canker. A preliminary bulletin. Fla. Agr. Exp. Sta. Bul. 122, p. 113–118, fig. 43–46.
(17) Swingle, W. T.
1915. A new genus, Fortunella, comprising four species of kumquat oranges. *In* Jour. Wash. Acad. Sci., v. 5, no. 5, p. 165–176, 5 fig.
(18) U. S. Department of Agriculture.
1915. Citrus canker in Philippines. *In* U. S. Dept. Agr. Dept. Circ., v. 1, no. 1, p. 8.
(19) Wolf, F. A., and Massey, A. B.
1914. Citrus canker. Ala. Agr. Exp. Sta. Circ. 27, p. 97–102, illus.

PLATE VIII

Fig. 1.—Grapefruit leaf showing young Citrus cankers.
Fig. 2.—Old Citrus canker on Satsuma leaves.
Fig. 3, 4.—Seedling grapefruit branches affected with Citrus canker.
Fig. 5.—Severe canker infection of branches of *Citrus trifoliata*.


1
2
3
4
5

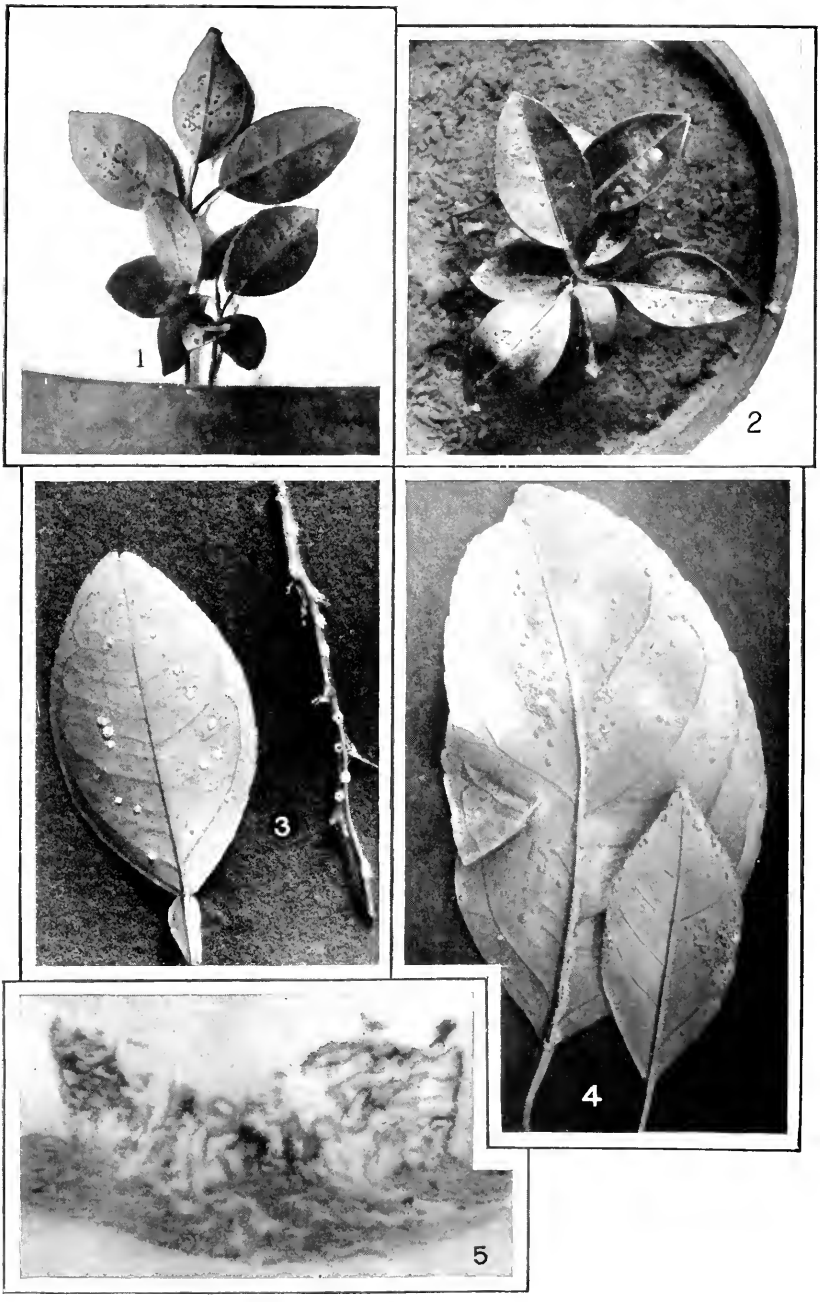

1
2
3
4
5

PLATE IX

Fig. 1.—View of lower side of leaves of seedling grapefruit artificially inoculated with *Pseudomonas citri.*

Fig. 2.—Top view of plant shown in figure 1.

Fig. 3.—Spongy white cankers on leaf and twig of seedling grapefruit produced by artificial inoculation. The plants were continuously kept under a bell jar in a humid atmosphere.

Fig. 4.—Citrus canker on Satsuma leaves resulting from artificial inoculation with *Pseudomonas citri.*

Fig. 5.—Photomicrograph of section of young, open canker on grapefruit.

PLATE X

Fig. 1.—Natural Citrus canker infection on leaves of *Citrus trifoliata*.

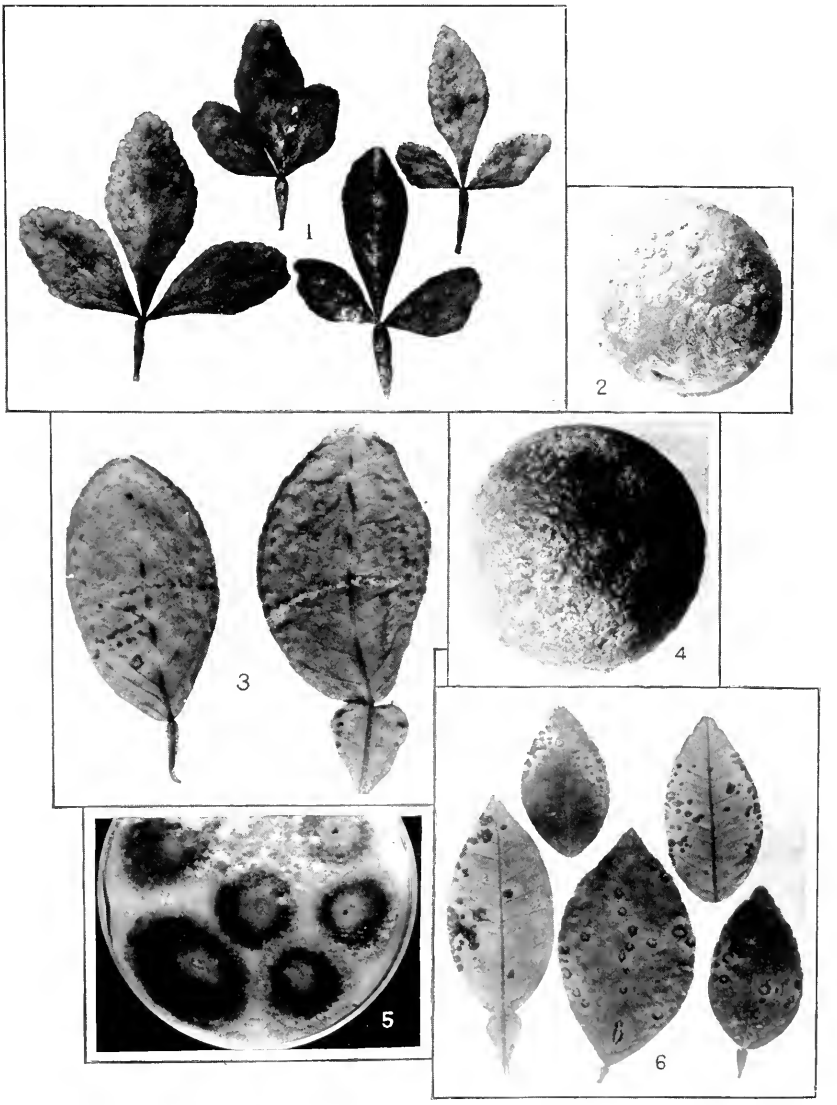
Fig. 2.—Mature cankers on fruit of *Citrus decumana* (courtesy of Dr. E. W. Berger).

Fig. 3.—Canker on seedling grapefruit leaves, entrance having been effected through abrasions made by thorns.

Fig. 4.—Young spongy cankers on fruit of *Citrus decumana*.

Fig. 5.—*Phoma socia* on cellulose agar showing dissolution of cellulose.

Fig. 6.—Mature cankerous areas on leaves of Duncan grapefruit.


1
2
3
4
5
6

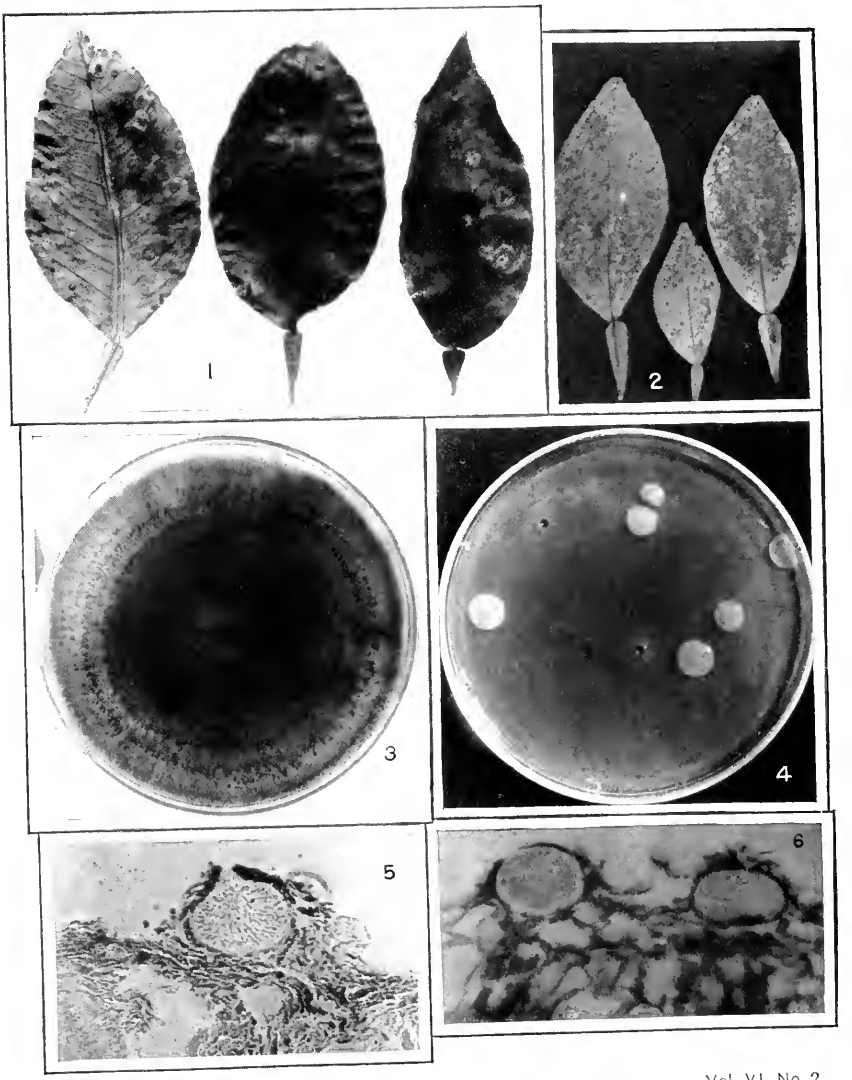

1
2
3
4
5
6

PLATE XI

Fig. 1.—Cankers on old grapefruit leaves which have enlarged during the second growing season.

Fig. 2.—Citrus canker resulting from immersion of leaves in a bacterial suspension. Lesions involving a large part of the lower leaf surface are thus formed.

Fig. 3.—Culture of *Phoma socia* showing pycnidial formation in concentric rings.

Fig. 4.—Dilution poured plate of *Pseudomonas citri* on green-bean agar. The spots on the colonies are the reflection of the windows of the room in which the exposure was made. Colonies 14 days old, the last 5 of which days the plates were kept in an ice chest at a temperature of about 55°.

Fig. 5.—Photomicrograph of pycnidium of *Phoma socia* taken in reflected sunlight.

Fig. 6.—Photomicrograph of pycnidia of *Phoma socia* taken in diffuse light.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

15 CENTS PER COPY

▽

Vol. VI APRIL 17, 1916 No. 3

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., APRIL 17, 1916 NO. 3

DETERMINATION OF STEARIC ACID IN BUTTER FAT[1]

By E. B. HOLLAND, *Associate Chemist*, and J. C. REED and J. P. BUCKLEY, Jr., *Assistant Chemists, Massachusetts Agricultural Experiment Station*

INTRODUCTION

Oils and fats are composed largely of neutral glyceryl esters together with small amounts of free fatty acids and unsaponifiable matter. Formerly the esters were considered simple glycerids, compounds of glycerol and three radicals of the same fatty acids. At present the opposite view seems to prevail and mixed glycerids are said to predominate in most products. The subject is controversial and difficult of solution. The constituents would be the same, however, in either case, whether combined as simple or complex molecules. The object of a technical examination of oils and fats is to isolate, identify, and determine the various fatty acids, glycerol, and unsaponifiable bodies, although, as Lewkowitsch asserts, this is not attainable in the present state of our knowledge. Certain progress has been made in determining different constituents of fats by indirect methods, such as iodin absorption, acetyl number, and molecular-weight calculations. Direct methods of fractional distillation, crystallization, and solubility of various salts have not, as a rule, proved sufficiently discriminative for quantitative use.

Fatty acids constitute about 95 per cent of most oils and fats and characterize the products to a large extent. The necessity of accurate methods for the quantitative determination of these acids has long been recognized not only from the standpoint of pure science but especially in physiological studies having as the object the measurement of the effect of different food groups on the production of body and milk fats. Many methods have been proposed since the publication of the work of Chevreul nearly 100 years ago, but few, if any, have met with general approval. After several years' investigations of the Partheil and Ferie method (7),[2] which proved unsatisfactory in the authors'[3] hands, a study of methods for determining stearic acid in butter fat was undertaken.

[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number to "Literature cited," p. 113.

[3] Mr. Reed was associated with the senior author in the earlier stages of the work and Mr. Buckley in the later.

EARLIER INVESTIGATIONS

For the separation of stearic from other fatty acids, David (1) recommended a special alcohol and dilute acetic-acid solution saturated with stearic acid at 15° C., in which solution oleic acid was shown to be soluble.

The Hehner and Mitchell (3) method for isolating stearic from other fatty acids was based on the hypothesis that a mixture of fatty acids heated with a solvent saturated at a given temperature with the acid under determination might be expected on cooling to that temperature to crystallize the whole of the acid sought, provided the other constituents did not increase the solubility. The solvent employed was methylated alcohol (94.4 per cent) saturated with stearic acid at 0.2° C., prepared by chilling a solution of 3 gm. to 1 liter overnight in ice water and siphoning off the saturated mother liquor through a small thistle tube covered with fine calico, using suction. The tests were conducted in a similar manner, taking from 0.5 to 5 gm. of insoluble acids (according to content) to 100 c. c. of alcohol-stearic-acid solution. Shaking was found to increase precipitation. Supersaturation and esterification were recognized as possible sources of error. The method gave concordant results with solid fats containing considerable stearic acid, but slight, if any, precipitate from the acids of butter fat and from mixtures of the acids of Japan wax and pure stearic acid.

Emerson (2) noted considerable variation in the content of different saturated solutions and found that supersaturation seemed to occur when less than 0.7 gm. to 100 c. c. was employed in preparing the solution. The formation of ethyl ester appeared to be a source of error and to have increased the apparent solubility of the stearic acid.

Kreis and Hafner (5) showed that small amounts of stearic acid below 0.1 gm. to 100 c. c. of a saturated solution formed supersaturated solutions, and that less than 0.05 gm. gave low and extremely variable results, even upon the addition of crystals of stearic acid.

Lewkowitsch (6, p. 556–559) claimed that the method yielded capricious results with mixtures of stearic, palmitic, and oleic acids, and that in many cases the results were entirely unreliable when other acids were present. He stated that a considerable proportion of lauric acid would prevent the complete precipitation of stearic acid, even when supersaturated alcohol-stearic-acid solutions were used, and that acids of higher melting point, when present, such as arachic, behenic, etc., would appear in the separated acids. He reported a precipitate of 0.49 per cent from butter fat, of which a portion might be arachic and myristic acids.

The results obtained by various investigators indicate that the solubility of stearic acid increases with the strength of the alcohol, but the figures reported are too variable to warrant further deductions (Table I).

TABLE I.—*Solubility of stearic acid, according to various investigators*

Investigator.	Approximate strength of alcohol.	Stearic acid to 100 c. c.	Saturation of 100 c. c. at 0° C.
	Per cent.	*Gm.*	*Gm.*
Hehner and Mitchell (3, p. 323)	94.4	0.2 to 0.5	0.1400 to 0.1580
Emerson (2, p. 1754)	95.5	.7	.1223
Do	95.1	.7	.1139
Do	94.5	.7	.1035
Kreis and Hafner (5)	95	.5	.1220 to .1310
Lewkowitsch (6, p. 164)	94.4	.3	.0814
Do	94.4	.7	.0810 to .1082
Ruttan (8, p. 440)	100		.373

PRELIMINARY WORK

In view of what has been stated, the outlook for another investigation was not promising, although Lewkowitsch's final arraignment of the process was not published until nearly a year after the work was undertaken. The subject was of sufficient importance, however, to warrant additional study whatever the outcome.

APPARATUS.—To insure a uniform temperature for crystallization, a tank was constructed of ⅞-inch lumber (20 inches long, 10 inches wide, and 20 inches deep), lined with galvanized iron, provided with a tight cover, and raised by legs to a convenient working height. For icing, a basket (13½ by 6 by 18 inches) of galvanized screening of $\frac{5}{16}$-inch mesh, holding probably 30 pounds of broken ice, was found very satisfactory. The insulation of wood, together with the large volume of water and ice, proved inadequate to meet the requirements of the case, and it was necessary to install in one corner of the tank a pump run by a motor, to keep the water in continuous circulation. With this apparatus a constant temperature of about 0.1° C. was easily maintained (fig. 1, 2).

Several factors had to be considered in the selection of containers in which the tests were to be conducted. They must be of a form, size, and weight suitable for weighing the charge on analytical balances, easily held in position in the tank, and such that the alcoholic solution could be removed while still in the tank, leaving the crystalline residue. After numerous experiments with globe-shaped separatory funnels and filtering tubes, 8-ounce sterilizer bottles were adopted and have been found fairly satisfactory. The bottles are of narrow cylindrical form (2 by 6¾ inches) and are held in place in the tank by pockets of wire screening, with only the rubber stopper and a small portion of the neck projecting out of the water. The solution is siphoned off by means of a small thistle tube (¼-inch bulb) having a felt of absorbent cotton weighing 0.020 gm. supported by a glass bead and covered with a piece of batiste.

REAGENTS.—For the preparation of an alcohol-stearic-acid solution constituents of high quality were deemed essential for satisfactory work. The purification of alcohol had been a subject for study for a number of years in connection with the ordinary analysis of oils and fats, and excellent results were finally secured by treatment with silver nitrate and caustic lime and redistillation. A strength of 95.25 per cent proved a satisfactory solvent for fatty acids, and greater strength was not considered necessary or even advisable.

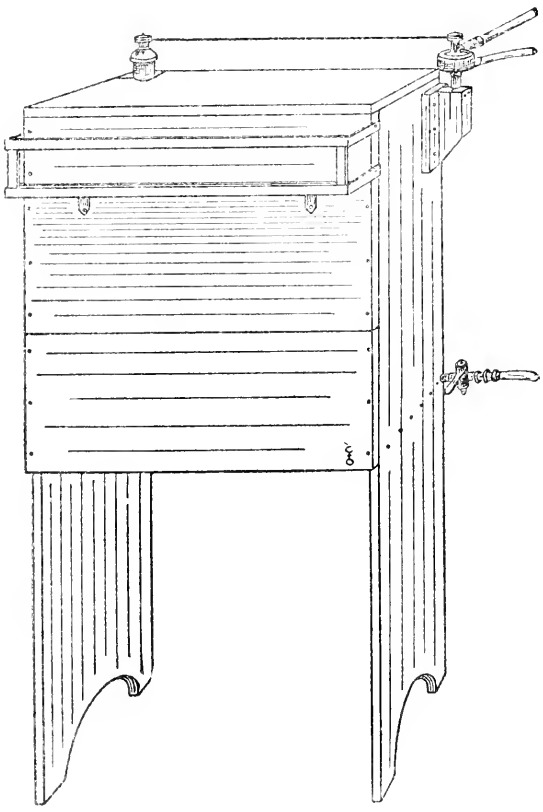


FIG. 1.—Exterior of constant-temperature crystallization tank.

One lot of stearic acid, a mixture of several grades, was purified by fractional distillation of the ethyl ester in vacuo and subsequent repeated crystallization of the separated acids from alcohol as previously described (4). Another lot of acid with a molecular weight of 271.13 was purified by 10 or more crystallizations from alcohol to a molecular weight of 284.25, and a second portion to 284.71, although the resulting leaflets were less perfect than those obtained by the former process.

When using separatory funnels and filtering tubes, alcohol-stearic-acid solutions, saturated at 0.1° C., applied to the insoluble acids of butter at the rate of 150 c. c. to 0.5 gm. of material, seldom yielded an appreciable amount of precipitate on standing, even with the addition of crystals of stearic acid and thorough agitation. Solutions testing about 0.22 and 0.24 gm. of stearic acid to 150 c. c. gave somewhat higher results, although of erratic and untrustworthy character. In the attempt to develop a method with this apparatus, over 140 determinations were made on butter acids, stearic acid, mixtures of butter and stearic acids, stearic and oleic acids, and stearic, myristic, and oleic acids. The object was not attained, and most of the data will be omitted, as

they would serve no useful purpose, merely indicating the time and labor involved. The results, however, with solutions of stearic acid appear to warrant certain deductions.

Solutions containing from 0.25 to 0.29 gm. of stearic acid to 150 c. c. crystallized, leaving a mother liquor of unlike composition (saturation).

The saturation varied inversely with the quantity of stearic acid present.

Presumably, therefore, supersaturation occurred as a result of insufficient stearic acid (Table II).

The time of standing may have had some influence, but when in excess of 24 hours it was of minor consequence.

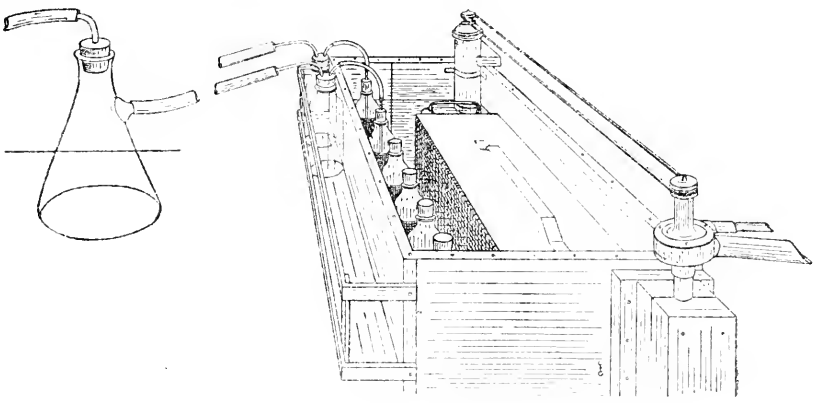


FIG. 2.—Interior of constant-temperature crystallization tank.

The form of the container as viewed in the light of subsequent work was a factor of some importance; a globe-shaped vessel was less effective than a narrow, cylindrical one of large surface.

TABLE II.—*Crystallization of stearic acid from solutions of different content, using separatory funnels*

Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).
	Gm.	*Gm.*			*Gm.*	*Gm.*	
0. 2406	0. 0100	0. 0130	0. 1584	0. 2400	0. 0304	0. 0640	0. 1376
. 2406	. 0150	. 0254	. 1535	. 2400	. 0354	. 0733	. 1347
. 2406	. 0150	. 0315	. 1494	. 2400	. 0475	. 0872	. 1335
. 2406	. 0400	. 0859	. 1298	. 2400	. 0481	. 0910	. 1314
. 2406	. 0450	. 0995	. 1241	. 2400	. 0491	. 0910	. 1321
. 2400	. 0200	. 0426	. 1449	. 2400	. 0498	. 0960	. 1292
. 2400	. 0251	. 0544	. 1405				

Stearic-acid solutions were found to crystallize more readily and with greater uniformity in sterilizer bottles than in separatory funnels, probably owing to the more rapid chilling of the narrow column of liquid and more thorough filtration.

Table III shows the amount of stearic acid crystallized from solutions of different content and the saturation of the mother liquor.

TABLE III.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

Alcohol.	Stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).	Alcohol.	Stearic acid taken.	Precipitate.	Saturation (grams in 100 c. c.).
C. c.	*Gm.*	*Gm.*		*C. c.*	*Gm.*	*Gm.*	
150	0. 2000	0. 0000	.....	150	0. 3670	0. 1880	0. 1193
150	. 2400	. 0020	0. 1587	150	. 3800	. 2000	. 1200
150	. 2705	. 0485	. 1480	150	. 4000	. 2210	. 1193
150	. 2815	. 0700	. 1410	150	. 4080	. 2260	. 1213
150	. 3055	. 1110	. 1297	150	. 4200	. 2435	. 1177
150	. 3215	. 1280	. 1290	150	. 4650	. 2980	. 1113
150	. 3475	. 1680	. 1197	150	. 5000	. 3255	. 1163
150	. 3600	. 1815	. 1190	150	. 6000	. 4315	. 1123

TABLE IV.—*Crystallization of stearic acid from solutions of different content, using sterilizer bottles*

Alcohol-stearic-acid solution (0.3990 gm. in 150 c. c.).	Alcohol.	Equivalent in stearic acid (grams in 150 c. c.).	Precipitate.	Saturation (grams in 100 c. c.).
	C. c.		*Gm.*	
100	50	0. 2660	0. 0555	0. 1403
110	40	. 2926	. 0980	. 1297
120	30	. 3192	. 1500	. 1128
130	20	. 3458	. 1745	. 1142
140	10	. 3724	. 2055	. 1113
150	0	. 3990	. 2335	. 1103

APPLICATION OF CRYSTALLIZATION METHOD

The facility with which alcohol-stearic-acid solutions crystallize increased with the concentration. Solutions of 0.40 to 0.45 gm. to 150 c. c. formed crystals readily, gave a satisfactory amount of precipitate, and when applied to the insoluble acids of butter yielded an additional amount from that source. This would indicate that if the stearic-acid content of the solution is sufficient, crystallization of stearic from butter acids is no more difficult than from other products. The results were very concordant for a crystallization method when all details of manipulation were strictly observed: The water maintained at the required level, properly iced at all times, and the pump run continuously at good speed. A gentle agitation of the solution after standing overnight in the ice tank assisted in completing the precipitation, but anything in

the nature of shaking reduced the fragile crystals to a mass and rendered filtration extremely difficult or impossible.

EXPERIMENTAL METHOD IN DETAIL

Five-tenths of a gram of melted insoluble acids are placed in an 8-ounce sterilizer bottle and 150 c. c. of an alcohol-stearic-acid solution (3 gm. to 1,000 c. c.), accurately measured with a pipette at 30° C., added. The bottle is sealed with a solid-rubber stopper, shaken at a gradually increasing temperature until a clear solution is obtained, placed immediately in a pocket of the ice tank, and allowed to stand overnight. The following morning the solution is gently agitated by inverting the bottle several times, and in the afternoon it is siphoned off as thoroughly as possible by means of a small thistle tube and a perforated rubber stopper, using suction. The residue is dissolved in ethyl ether, transferred to a tared 140 c. c. wide-mouth Erlenmeyer flask, the ether carefully distilled off, the residue dried at 100° C., and weighed. As saturation may vary somewhat with the amount of stearic acid present and as the quantity of solution retained by the precipitate depends in a measure on the amount of precipitate, blanks are run on a weight of stearic acid equivalent to that expected in the test. By deducting the additional stearic acid taken from the weight recovered the true blank for the alcohol-stearic-acid solution is obtained.

NATURE OF THE PRECIPITATE

To ascertain whether the crystalline substance obtained from butter acids was stearic acid or a mixture, the residues from a number of tests (one being insufficient for accurate work) were combined and the molecular weight determined by saponification. Such a determination made after securing satisfactory control of the stearic-acid method gave 284.64, theoretically 284.288. The melting point was not determined, as it was considered less reliable than the molecular weight.

INFLUENCE OF DIFFERENT FATTY ACIDS ON PRECIPITATION OF STEARIC ACID

Numerous tests were made in an effort to determine whether lauric, myristic, palmitic, and oleic acids had any effect on the crystallization of stearic acid and, if so, the nature and extent of such action. Table V will serve to illustrate.

According to molecular-weight determinations the lauric and palmitic acids were of excellent quality and the myristic and oleic acids somewhat inferior.

Lauric, myristic, and oleic acids in relatively large amounts showed no appreciable influence on the crystallization of stearic acid. Palmitic acid, on the other hand, noticeably increased the solubility and affected the crystalline structure of the precipitate.

TABLE V.—*Effect of different fatty acids on precipitation of stearic acid*

Alcohol-stearic acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Other acids taken.	Precipitate.	Saturation (grams in 100 c. c.).
	Gm.	*Gm.*	*Gm.*	
STEARIC ACID				
0.3990	0.1000	.....	0.3420	0.1047
.3990	.1015	.....	.3430	.1050
.3990	.1035	.....	.3415	.1073
.3990	.1000	.....	.3405	.1057
LAURIC ACID				
0.3990	.1030	0.4000	.3455	.1043
.3990	.1000	.4000	.3415	.1050
.3990	.1000	.4000	.3430	.1040
.3990	.1010	.4000	.3450	.1033
MYRISTIC ACID				
0.3990	.1000	.4000	.3495	.0997
.3990	.1010	.4000	.3480	.1013
.3990	.1000	.4000	.3490	.1000
.3990	.1000	.4000	.3515	.0983
PALMITIC ACID				
0.3990	.1055	.4000	.3135	.1273
.3990	.1000	.4030	.2980	.1340
.3990	.1010	.2500	.2965	.1357
.3990	.1040	.2500	.3065	.1310
.3990	.1050	.2000	.3085	.1303
OLEIC ACID				
0.3990	.1070	.4220	.3515	.1030
.3990	.1010	.4255	.3440	.1040
.3990	.1000	.4000	.3485	.1003
.3990	.1035	.4000	.3460	.1043

The addition of palmitic acid to butter acids reduced the amount of stearic acid recovered in the test. Some of our more recent determinations indicated that the solvent action of palmitic acid can be counteracted in a large measure, if not entirely, by increasing the relative amount of stearic acid in solution. With butter acids of average palmitic acid content, an alcohol-stearic-acid solution, containing at least 3 gm. of stearic acid to the liter, is necessary and possibly 3.4 or 3.7 gm. may prove more reliable. This, however, seems to depend to a considerable degree upon the alcohol-stearic-acid solution employed. Some solutions made

from purified alcohol of approximately the same strength require more stearic acid than others to insure a constant saturation, the reason for which we have been unable as yet to determine. Some of the results cited in Tables VI to VIII are probably low, owing to insufficient stearic acid in solution, although the results are all calculated with reference to blank tests conducted under precisely like conditions.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution A (0.8153)[a]	.....	0.3990	0.0525	0.2900	0.2375	0.1077	.....
	.....	.3990	.0500	.2880	.2380	.1073	.....
	.....	.3990	.0500	.2895	.2395	.1063	.....
					[b] .2383		
Solution B (0.8135)[a]	.....	.3960	.0515	.2630	.2115	.1230	.....
	.....	.3960	.0530	.2640	.2110	.1233	.....
	.....	.3960	.0505	.2625	.2120	.1227	.....
	.....	.3960	.0490	.2605	.2115	.1230	.....
	.....	.3960	.0500	.2610	.2110	.1233	.....
					[b] .2114		
Solution C (0.8142)[a]	.....	.4050	.0575	.2770	.2195	.1237	.....
	.....	.4050	.0500	.2710	.2210	.1227	.....
	.....	.4050	.0800	.2990	.2190	.1240	.....
	.....	.4050	.0800	.3005	.2205	.1230	.....
					[b] .2200		
Solution D (0.8142)[a]	.....	.4050	.0820	.3040	.2220	.1220	.....
	.....	.4050	.0805	.3045	.2240	.1207	.....
					[b] .2230		
Solution E (0.8147)[a]	.....	.4470	.1115	.3765	.2650	.1213	.....
	.....	.4470	.1115	.3765	.2650	.1213	.....
					[b] .2650		
Solution F (0.8147)[a]	.....	.4440	.1105	.3840	.2735	.1137	.....
	.....	.4440	.1100	.3830	.2730	.1140	.....
					[b] .2733		
Solution A:							
4	.5440	.3990	.....	.2930	.2383	.....	10.06
4	.5170	.3990	.....	.2900	.2383	.....	10.00
4	.5235	.3990	.....	.2915	.2383	.....	10.16
4	.5000	.3990	.....	.2905	.2383	.....	10.44
							[b] 10.17
Solution B:							
5	.5085	.3960	.....	.2460	.2114	.....	6.80
5	.5190	.3960	.....	.2480	.2114	.....	7.05
							[b] 6.93
6	.5230	.3960	.....	.2555	.2114	.....	8.43
6	.5010	.3960	.....	.2520	.2114	.....	8.10
							[b] 8.27
7	.5135	.3960	.....	.2515	.2114	.....	7.81
7	.5230	.3960	.....	.2500	.2114	.....	7.38
							[b] 7.60

[a] Hydrometer reading at 15.56° C. of the alcohol employed. [b] Average.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
Solution A:	*Gm.*		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
8.....	0.5170	0.3990	.....	0.2850	0.2383	.....	9.03
8.....	.5120	.3990	.....	.2840	.2383	.....	8.93
8.....	.5225	.3990	.....	.2830	.2383	.....	8.56
							[a] 8.84
Solution C:							
9 [b].....	.5090	.4050	.....	.2970	.2200	.....	15.13
9.....	.5155	.4050	.....	.3000	.2200	.....	15.52
9.....	.5150	.4050	.....	.2970	.2200	.....	14.95
9.....	.5130	.4050	.....	.2980	.2200	.....	15.20
							[a] 15.20
Solution B:							
10 [b].....	.5130	.3960	.....	.3000	.2114	.....	17.27
10.....	.5050	.3960	.....	.3020	.2114	.....	17.94
Solution C:							
10 [b].....	.4995	.4050	.....	.3070	.2200	.....	17.42
10.....	.5060	.4050	.....	.3075	.2200	.....	17.29
10.....	.5255	.4050	.....	.3140	.2200	.....	17.89
							[a] 17.56
Solution B:							
11 [b].....	.5065	.3960	.....	.2990	.2114	.....	17.30
11.....	.5150	.3960	.....	.3000	.2114	.....	17.20
							[a] 17.25
Solution C:							
14.....	.5165	.4050	.....	.2645	.2200	.....	8.62
14.....	.5070	.4050	.....	.2635	.2200	.....	8.58
14.....	.5015	.4050	.....	.2625	.2200	.....	8.47
							[a] 8.56
15.....	.5045	.4050	.....	.2705	.2200	.....	10.01
15.....	.5060	.4050	.....	.2715	.2200	.....	10.18
							[a] 10.10
16.....	.5265	.4050	.....	.2665	.2200	.....	8.83
16.....	.5205	.4050	.....	.2680	.2200	.....	9.22
							[a] 9.03
17 [c].....	.5045	.4050	.....	.2965	.2200	.....	15.16
17.....	.5300	.4050	.....	.2980	.2200	.....	14.72
							[a] 14.94
18 [c].....	.5035	.4050	.....	.2945	.2200	.....	14.80
18.....	.4990	.4050	.....	.2930	.2200	.....	14.63
18.....	.5105	.4050	.....	.2940	.2200	.....	14.50
							[a] 14.64
19 [c].....	.5205	.4050	.....	.2925	.2200	.....	13.93
19.....	.5110	.4050	.....	.2905	.2200	.....	13.80
							[a] 13.87
Solution D:							
20.....	.5180	.4050	.....	.2720	.2230	.....	9.46
20.....	.5025	.4050	.....	.2715	.2230	.....	9.65
							[a] 9.56
21.....	.5090	.4050	.....	.2630	.2230	.....	7.86
21.....	.5205	.4050	.....	.2650	.2230	.....	8.07
							[a] 7.97

[a] Average. [b] The cows were fed beef tallow. [c] The cows were fed palm oil.

TABLE VI.—*Amount of stearic acid in the insoluble acids of butter fat*—Continued

Sample No.	Insoluble acids of butter taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution F:							
I	0.5070	0.4440	.....	[a]0.3850	0.2733	.....	22.03
I	.5225	.4440	.....	.3915	.2733	.....	22.62
							[b]22.33
II	.5205	.4440	.....	[a].3830	.2733	.....	21.08
II	.5215	.4440	.....	.3850	.2733	.....	21.42
							[b]21.25
Solution E:							
III	.5090	.4470	.....	[c].3525	.2650	.....	17.19
III	.5140	.4470	.....	.3525	.2650	.....	17.02
III	.5060	.4470	.....	.3535	.2650	.....	17.49
							[b]17.23
IV	.5015	.4470	.....	[c].3505	.2650	.....	17.05
IV	.5035	.4470	.....	.3520	.2650	.....	17.28
							[b]17.17

[a] Molecular weight of the several precipitates, 284.54.
[b] Average.
[c] Molecular weight of the several precipitates, 284.59.

TABLE VII.—*Amount of stearic acid in the insoluble acids of beef tallow*

Sample No.	Insoluble acids of beef tallow taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution A	.....	0.3990	0.1500	0.3870	0.2370	0.1080	.....
Do	.....	.3990	.1555	.3930	.2375	.1077	.....
					[a].2373		
Solution B	.....	.3960	.1520	.3690	.2170	.1193	.....
Do	.....	.3960	.1550	.3700	.2150	.1207	.....
					[a].2160		
Solution A:							
1	0.5280	.3990	.....	.3975	.2373	.....	30.34
1	.5155	.3990	.....	.3960	.2373	.....	30.79
							[a]30.57
Solution B:							
2	.5025	.3960	.....	.3740	.2160	.....	31.44
2	.5150	.3960	.....	.3775	.2160	.....	31.36
							[a]31.40

[a] Average.

TABLE VIII.—*Amount of stearic acid in the insoluble acids of palm oil*

Sample No.	Insoluble acids of palm oil taken.	Alcohol-stearic-acid solution (grams in 150 c. c.).	Additional stearic acid taken.	Precipitate.	Blank.	Saturation (grams in 100 c. c.).	Stearic acid.
	Gm.		*Gm.*	*Gm.*	*Gm.*		*Per cent.*
Solution C		0.4050	0.1515	0.3745	0.2230	0.1213	
Do		.4050	.1500	.3750	.2250	.1200	
Do		.4050	.2000	.4255	.2255	.1197	
Do		.4050	.2030	.4295	.2265	.1190	
					[a].2250		
Solution C:							
12	0.3405	.4050	.1500	[b].4040	.2250		8.52
12	.4110	.4050	.1510	.4100	.2250		8.27
12	.5205	.4050	.1540	.4265	.2250		9.13
12	.5000	.4050	.1500	.4205	.2250		9.10
12	.5215	.4050	.1500	.4245	.2250		9.49
12	.5135	.4050	.1565	.4275	.2250		8.96
							[a] 8.91

[a] Average. [b] Molecular weight of the several precipitates, 284.38.

The stearic acid obtained from the insoluble acids of butter fat by the method described ranges from 7 to 22 per cent, which is considerably in excess of the amount generally credited to the product. The prevailing opinion was supported undoubtedly by the fact that only a small amount of precipitate is obtainable by the Hehner and Mitchell (3) method, as shown by several investigators.

The amount of stearic acid appears to be affected by the feed the animal receives. Samples 9, 10, and 11, averaging 16.67 per cent, were from cows fed beef tallow; samples 17, 18, and 19, averaging 14.48 per cent, were from those fed palm oil; while samples 4 to 8, 14 to 16, 20 and 21, averaging 8.70 per cent, were from those fed a ration low in fat. It is probable that the individuality of the animal and period of lactation also affect the composition. The entire matter of the effect of food as well as other influences upon the chemical character of butter fat is now being further studied.

The stearic acid (8.91 per cent) recovered from the insoluble acids of palm oil exceeded the amount usually reported.

SUMMARY

The results of the determinations of stearic acid in the insoluble acids of butter fat by the method proposed show a higher percentage of stearic acid than has been generally reported. The facts that the results are concordant and that the molecular weight determinations of the crystallized product secured by the proposed method agree closely with the theoretical molecular weight leave no doubt as to the identity and approximate purity of the stearic acid.

LITERATURE CITED

(1) DAVID, J.
1878. Méthode de dosage et de séparation de l'acide stéarique et de l'acide oléique provenant de la saponification des suifs. *In* Compt. Rend. Acad. Sci. [Paris], t. 86, no. 22, p. 1416–1418.

(2) EMERSON, W. H.
1907. The solubility of stearic acid in ethyl alcohol at zero. *In* Jour. Amer. Chem. Soc., v. 29, no. 12, p. 1750–1756.

(3) HEHNER, Otto, and MITCHELL, C. A.
1896. On the determination of stearic acid in fats. *In* Analyst, v. 21, no. 249, p. 316–332, 1 fig.

(4) HOLLAND, E. B.
1911. Purification of insoluble fatty acids. *In* Jour. Indus. and Engin. Chem., v. 3, no. 3, p. 171–173.

(5) KREIS, Hans, and HAFNER, August.
1903. Über Stearinsäure-Bestimmungen. *In* Ztschr. Untersuch. Nahr. u. Genussmtl., Jahrg. 6, p. 22–27.

(6) LEWKOWITSCH, J.
1913. Chemical Technology and Analysis of Oils, Fats, and Waxes. Ed. 5, v. 1. London.

(7) PARTHEIL, A., and FERIÉ, F.
1903. Zur Kenntnis der Fette. *In* Arch. Pharm., Bd. 241, Heft 7, p. 545-569, illus.

(8) RUTTAN, R. F.
1913(?) Margaric acid and its relation to palmitic & stearic acids. *In* Orig. Com. 8th Internat. Cong. Appl. Chem., v. 25, 1912, p. 431–442.

LIFE HISTORY AND HABITS OF TWO NEW NEMATODES PARASITIC ON INSECTS[1]

[PRELIMINARY PAPER]

By J. H. MERRILL, *Assistant Entomologist in Charge of Fruit-Insect Control*, and A. L. FORD, *Assistant in Life-History Studies, Kansas State Agricultural Experiment Station*

INTRODUCTION

While investigating the life history and methods of control of the elm borer (*Saperda tridentata* Oliv.) and the termite (*Leucotermes lucifugus* Rossi) at the Kansas Agricultural Experiment Station, two new nematodes were found, one parasitic on the former and the other parasitic on the latter. One hundred and twenty-one adult beetles obtained from one tree[2] were placed in breeding cages, but in no instance were eggs deposited, and both sexes eventually weakened and died. Examination after death showed that the intestines were so filled with nematodes that in only one female were eggs even developed in the body. The death rate due to nematode parasitization was apparently 100 per cent. Several colonies of *Leucotermes lucifugus* were placed in salve boxes, together with food. Inasmuch as *Saperda tridentata* had shown so high a nematode parasitization, it was naturally suggested that nematodes might be present in the termites. Accordingly a number of these insects were killed and examined, with the result that nematodes were found infesting the head in varying degrees. Of the colonies taken, 76.92 per cent were parasitized with nematodes. The parasitism of the individuals in single colonies ranged from 0 to 100 per cent.

DIPLOGASTER LABIATA

The nematodes were submitted to Dr. N. A. Cobb, of the Bureau of Plant Industry, United States Department of Agriculture, for identification. He found that the nematode parasitizing *Saperda tridentata* was a new species which he named "*Diplogaster labiata*" (fig. 1; 2, *A–H*), and described as follows:

Diplogaster labiata, n. sp. $\frac{1.2\ \ 17.\ \ 21.\ \ 59'^{48}\ \ 91.}{2.1\ \ 4.2\ \ 4.2\ \ 4.4\ \ 2.9}$ 0.66 mm. (The formula was derived from a single specimen.) The thin layers of the transparent, colorless, naked cuticle are traversed by fine transverse striæ, resolvable with high powers into rows of dots, more particularly near the head and on the tail, those on the tail being somewhat irregularly placed. The cuticle is also longitudinally striated, and the dots of the transverse striations are coincident with those of the longitudinal striations. The longi-

[1] Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 17. This paper embodies the results of some of the investigations undertaken by the authors in the prosecution of projects Nos. 13 and 101, Kansas Agricultural Experiment Station.

[2] A tent was placed around an elm tree so that all emerging insects might be secured for breeding purposes.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dd

Vol. VI, No. 3
Apr. 17, 1916
Kans.—2

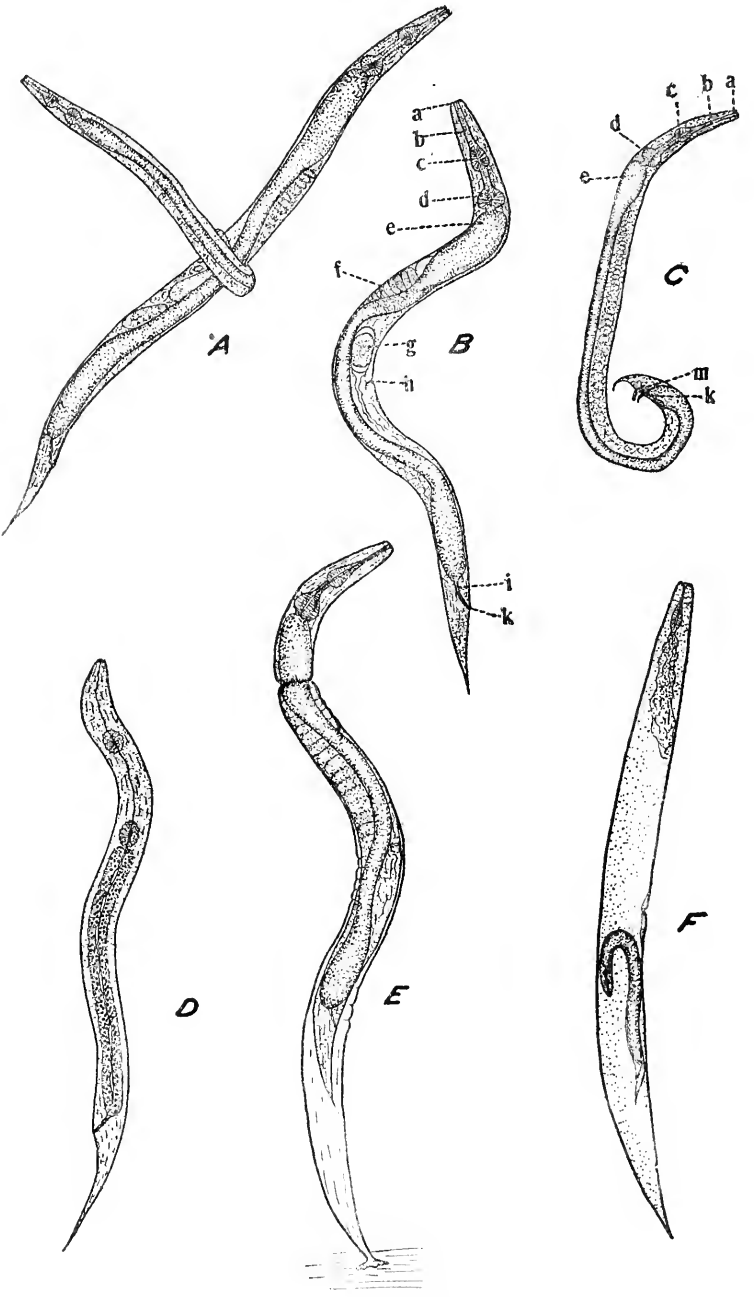


FIG. 1.—*Diplogaster labiata:* *A*, Mating (× 125); *B*, mature female reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *g*, egg, *h*, genital pore, *i*, rectum, *k*, anus; *C*, mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *D*, at time of hatching (× 400); *E*, female during process of molting (× 125); *F*, dead female with young nematode which hatched within her body (× 125). Drawings by A. L. Ford.

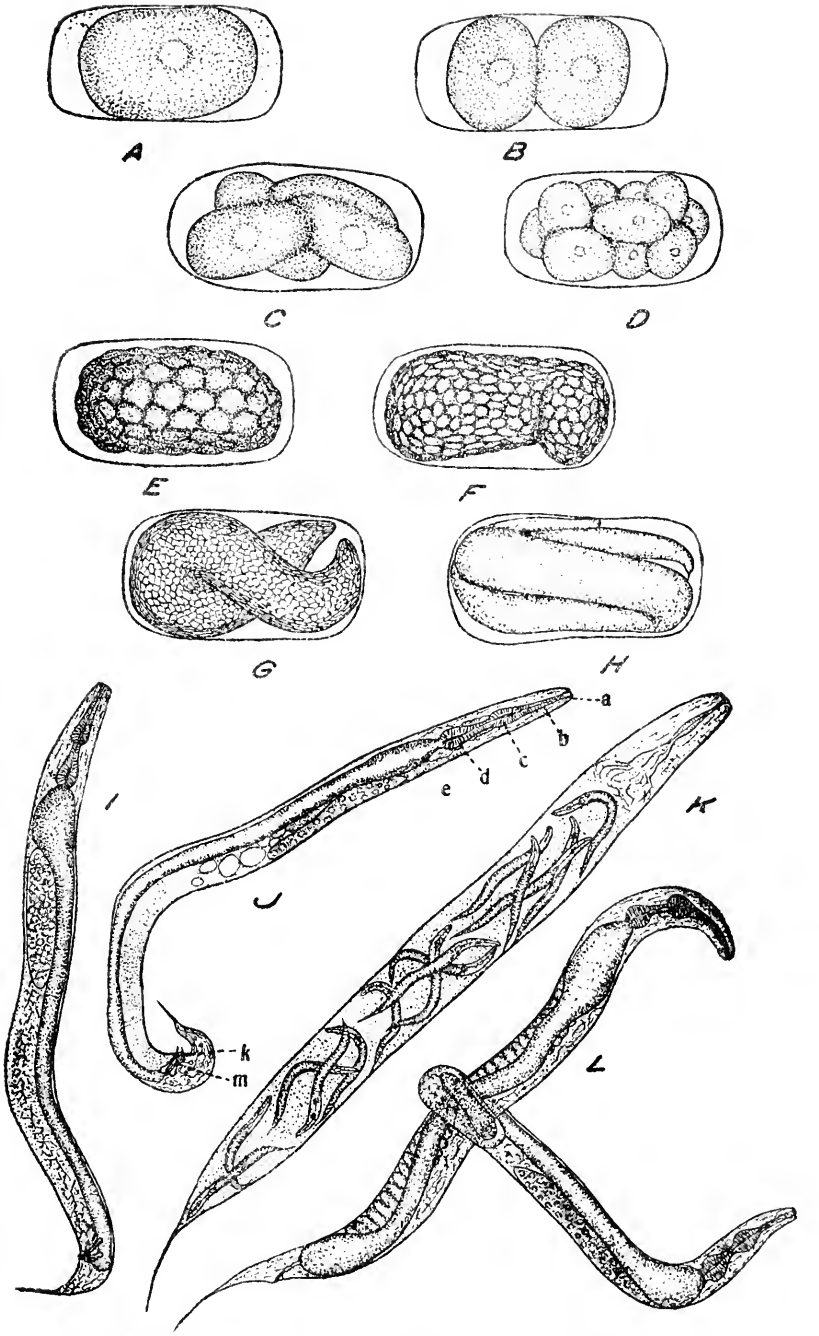


FIG. 2.—*A–H, Diplogaster labiata:* Development of the egg (× 500); *I*, Diplogaster aerivora: mature male reared in moist soil (× 160); *J, Diplogaster aerivora:* mature male reared in water culture (× 125), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *k*, anus, *m*, spicula; *K, Diplogaster aerivora:* dead female with young which hatched within her body (× 125); *L, Diplogaster aerivora:* mating (× 125). Drawings by A. L. Ford.

tudinal striæ are not present on the lateral fields, this naked space being one-third to one-half the width of the body. The slightly conoid neck becomes slightly convex-conoid near the head, the lip region of which is set off by a very broad, almost imperceptible constriction. There are six strongly developed and fairly distinct lips, each ending in a conoid tip, from the summit of which issues a very short innervated bristle-like papilla. The lips have a more or less distinct refractive framework and are in all probability quite mobile. Usually in specimens which have been fixed in Flemming's solution the tips of the lips are slightly outward-pointing, leaving a somewhat circular refractive mouth opening about two-fifths as wide as the front of the head. The inner surface of the lips is so strongly refractive that usually the posterior limits of the lips are distinctly visible, more particularly as the wall of the pharynx at this point is encircled by a very delicate refractive line lying considerably in front of the middle of the pharynx. This latter appears to be irregularly cylindroid, but is slightly unsymmetrical at the base. On the whole, it is about two-fifths as wide as the head. It appears to possess at the base a rather well-developed but blunt, slightly inward-projecting process or tooth. In the lateral view, as the posterior part of the pharynx appears to pass around this projection, it acquires the slightly unsymmetrical contour already mentioned. The walls of the esophagus are rather distinctly ceratinized. The esophagus begins at the base of the pharynx as a tube two-thirds as wide as the base of the head and continues to have this diameter, or a slightly greater, until it reaches a point halfway back to the median bulb. Thence onward it diminishes slightly, so that just in front of the median bulb it is only half as wide as the middle of the neck. The median bulb is a well-developed, elongated or ellipsoidal, radially muscular structure, with a somewhat distinct elongated but narrow valve. This bulb is about two-thirds as wide as the middle of the neck. Behind the median bulb the esophageal tube continues with a diameter one-third to two-fifths as great as the corresponding portion of the neck but diminishes very slightly, so that just in front of the ellipsoidal cardiac bulb it is less than one-third as wide as the corresponding portion of the neck. The cardiac bulb contains a rather distinct and rather complicated threefold valvular apparatus and is capable of opening out posteriorly, so that the lumen of the posterior part of the bulb, where it debouches into the intestine, then becomes one-fourth as wide as the corresponding portion of the body. The lining of the esophagus is a distinct feature throughout its length. The intestine, which is thin-walled at first, is separated from the esophagus by a distinct constriction. It becomes at once four-fifths to five-sixths as wide as the body and presents at the beginning a distinct cardiac cavity. There is also a distinct cardia. The cells of the intestine, which are of such size that probably four are required to build a circumference, contain rather large nuclei and are packed with granules of variable size, the largest of which have a diameter as great as the distance between two of the longitudinal striæ, the smallest of which are very much smaller. The lining of the intestine is refractive, so that the lumen is usually quite a distinct feature. From the slightly raised anus the narrow, refractive, ceratinized rectum, which is one and one-half to two times as long as the anal body diameter, extends inward and forward. The tail end begins to taper from some distance in front of the anus but in front of the anus tapers only very slightly. Behind the anus it tapers rather regularly to an acute point. Near the middle of the tail there appears to be a lateral papilla on each side. From the slightly raised, rather broad vulva the vagina leads inward at right angles to the ventral surface nearly halfway across the body, where it joins the two uteri, which extend in opposite directions. The reflexed ovaries reach more than halfway back to the vulva, at any rate in apparently young specimens in which no eggs exist in the uterus. The ova in the ovary are arranged more or less single file for about half its length; toward the blind end they are arranged irregularly. Fertilized females show sperm cells in the uterus of such a

size that about four to five side by side would span the body diameter. Numerous micro-organisms were seen in the intestine.

Male formula. $\frac{1.9\ \ 16.\ \ 21.\ \ 'M'^{59}.\ \ 94.}{1.7\ \ 3.1\ \ 3.5\ \ 3.9\ \ 2.9}$ 0.72 mm. (single specimen). The tail of the male differs materially in form from that of the female. It begins to taper at the anus, and it tapers rapidly in the anterior two-thirds, more particularly in the middle third, so that at the beginning of the final third it is only about one-tenth as wide as at the anus. Thence onward it tapers rather regularly to the exceedingly fine terminus; there is, however, a pronounced ventral elevation at the beginning of the small part of the tail, though it remains uncertain whether this elevation is innervated. The middle portion of the tail is strongly convex-conoid, the convexity existing largely on the dorsal side. The cuticle of the tail presents a peculiar arrangement of the dots, such that there is an appearance of two sets of oblique fibers crossing each other, these fibers being arranged approximately at 45° to the longitudinal lines. The two equal, rather uniform, somewhat arcuate, blunt spicula are about one and one-fourth to one and one-half times as long as the anal body diameter. Their proximal ends, which are slightly narrower than the main portion, are set off by a rather broad and prominent constriction. At their widest part, through the middle, they are about one-fifth to one-sixth as wide as the corresponding portion of the body. The accessory piece is about half as long as the spicula. It is very inconspicuous near the anus, but lies parallel to the spicula. It widens out to a somewhat clavate or elongated pyriform contour, and has its rounded proximal end toward the dorsal side of the body, and from this blunt end muscular fibers pass obliquely backward to the ventral surface of the tail and join the caudal wall at a distance nearly half way from the anus to the beginning of the narrow portion. Oblique copulatory muscles are to be seen opposite the ejaculatory duct for a distance about one and one-half times as great as the length of the tail. The male papillæ are arranged as follows: One ventrally submedian pair a little in front of the proximal ends of the spicula; one ventrally submedian pair a little in front of the anus, and one ventrally sublateral pair on the same zone; another sublateral pair just opposite the anus; a lateral pair slightly behind the middle of the enlarged portion of the tail; a submedian pair nearly halfway from that last mentioned to the beginning of the small part of the tail; a dorsally sublateral pair a little in front of the beginning of the narrow portion of the tail; three subventral pairs close together opposite that last mentioned; between the members of these three subventral pairs, possibly a single ventral papilla. The most pronounced of these papillæ can hardly be called digitate. The ejaculatory duct is about two-fifths as wide as the body. The vas deferens is nearly two-thirds as wide as the body. The testis tapers so that at the point of inflection, a short distance behind the cardiac bulb, it is about one-fourth as wide as the body. The blind end lies about two body widths behind the flexure.

Habitat: Manhattan, Kans., 1915, on *Saperda tridentata*.

The eggs of *Diplogaster labiata*, elliptical in shape, about twice as long as wide, with bluntly rounded ends, when freshly deposited, were uniformly dark brown or gray, but after segmentation began they became darker. Their average length was 0.0627 mm. and the average diameter 0.031 mm. They were laid singly with apparently no preference as to the place of deposition. Occasionally segmentation began before the eggs were deposited. From the beginning of segmentation the cell divisions could be plainly followed throughout (fig. 2, *A–H*).

A few hours before emerging, the folded young nematodes made slight movements within the egg. Later these movements became

more vigorous until finally they ruptured the shells and emerged, after which the egg walls collapsed. Occasionally a young nematode hatched within the body of a dead female. In cultures the eggs hatched in from 30 to 32 hours from the time of deposition, and the nematodes matured in from 7 to 10 days. The males appeared to mature slightly in advance of the females.

At hatching, the young nematodes were about 0.2 mm. in length (fig. 1, *D*), very slender, and sluggish, and remained for a time in a curled position. Later they straightened out their bodies and became very active. The young worms were almost transparent (in water cultures), there being no solid food in the alimentary canal. As development proceeded, the young became darker in color and more active. At the end of 5 days the sex organs began to appear, and in from 7 to 10 days the nematodes reached maturity.

Specimens which were isolated and kept under observation were noted to molt at least three times, these molts occurring about three days apart. The process of molting (fig. 1, *E*) was as follows: The nematode first fastened its posterior end to any surface upon which it might be resting. The skin then broke at the anterior end and the nematode began to emerge. At first the process was very slow, owing to the fact that the opening of the molt skin was smaller in diameter than the middle part of the body. By moving vigorously from side to side, the nematode slowly worked its way out of the skin. After the widest portion of the body had passed through the opening, no further resistance to emergence was offered, as the posterior end rapidly decreased in diameter. The nematodes were not always able to emerge, as occasionally specimens were found which died before completing the process. Molting lasted from 45 minutes to 6 hours.

The adults and the young were similar in form and food habits, but differed in that the adults possessed sex organs. The mature females were about 0.7 mm. in length and 0.03 mm. in diameter, while the males were about 0.6 mm. in length and 0.02 mm. in diameter.

As soon as maturity was reached, mating began (fig. 1, *A*). The male fastened its caudal end around the middle of the female's body. During this process the male held its body rigid, while the female moved vigorously from side to side. It was not uncommon to find males in the act of mating with their bodies wrapped twice about the females. Toward the end of the process the female increased her activity and soon shook the male free. Many matings were observed, the shortest of which lasted about 2 minutes and the longest 30 minutes.

PROPORTION OF SEXES.—Of 367 specimens examined, 229 were found to be females and 138 were males. In other cultures in which counts were not made the females were noticed to be more abundant than the males.

PERIOD OF OVIPOSITION.—While in the specimens of *Diplogaster labiata* under observation mating usually occurred but once, occasionally a few individuals mated a second time. Oviposition began from two to four hours after mating and lasted over a period of about two days, during which time the average number of eggs deposited was seven.

HABITS.—These nematodes infested the intestines of adults of *Saperda tridentata* in such large numbers that they prevented these insects from performing their natural functions. They lived in the alimentary canal in such large numbers that they ruptured the walls of the canal and, escaping into the body cavity of the insect, caused its death.

The examination of individuals of *Saperda tridentata* which had died in this manner rarely showed eggs that had started to develop. Specimens of *Diplogaster labiata* placed in water cultures were fed on macerated bodies of *Saperda tridentata*. They flourished on this, but since the supply was soon exhausted, substitute foods had to be used. Different substances were tried with varying success, but macerated beetles placed in water seemed to be the most satisfactory. Nematodes in cultures without food usually did not live longer than two days. The presence of food acted as a stimulant to copulation and oviposition, but both varied directly with the abundance and adaptability of the food.

The nematodes seemed to show no preference to either day or night for depositing their eggs or any other of their habits.

LENGTH OF ACTIVE BREEDING STATE.—If the nematode is considered to be mature from the time of mating, it spends an average of about two days as a normal active breeding adult.

DIPLOGASTER AERIVORA

In 1856, Charles Lespés[1] gave a meager description of a nematode which he found parasitizing *Leucotermes lucifugus*. His description is short and so indefinite that it might apply to several species of nematodes, but the habits he discusses closely resemble those of the nematodes found in *L. lucifugus* in Kansas. However, Dr. Cobb identified this nematode as *Diplogaster aerivora* (fig. 2, *I–L;* 3) and described it as follows:

Diplogaster aerivora, n. sp. $\frac{.8\ \ 8.9\ \ 12.\ \ '51^{162}\ \ 87.}{1.6\ \ 3.9\ \ 4.9\ \ 5.9\ \ 2.6}$ 1.5 mm. The transparent, moderately thin layers of the colorless naked cuticle are traversed by fine transverse striæ, resolvable with high powers under favorable conditions. The cuticle is traversed also by 24 longitudinal striæ. These longitudinal striæ are sometimes resolvable into quadrate elements, each consisting of four punctations arranged in a quadrangle whose width is equal to the width of the stria. In the majority of specimens these quadrate elements were not to be seen. The distance between the striæ varies in different parts of the body up to about twice their width. The striations of the cuticle, both transverse and longitudinal, vary within pretty wide limits, the varying

[1] Lespés, Charles. Sur un nématoïde parasite des Termites. *In* Ann. Sci. Nat. Zool., s. 4, t. 5, p. 335–336. 1856.

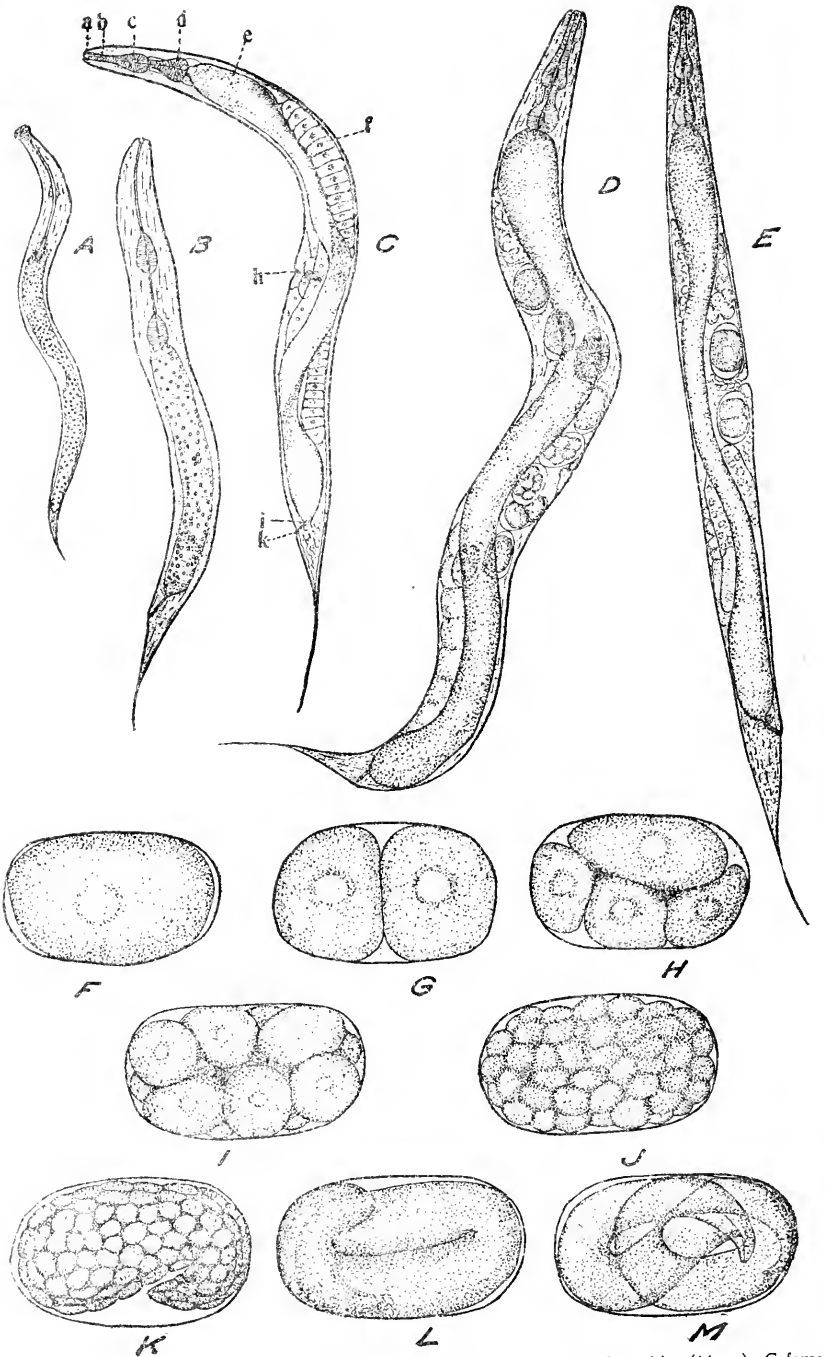


FIG. 3.—*Diplogaster aerivora:* *A*, Form found in termite (× 150); *B*, at time of hatching (× 400); *C*, female reared in water culture, not quite mature (× 100), *a*, lip region, *b*, esophagus, *c*, median bulb, *d*, cardiac bulb, *e*, intestine, *f*, ovaries, *h*, genital pore, *i*, rectum, *k*, anus; *D*, mature female reared in moist soil (× 75); *E*, mature female reared in water culture (× 125); *F–M*, development of the egg (× 500). Drawings by A. L. Ford.

conditions evidently being a function among other things of the age or condition of the cuticle. There are lateral wings, though these consist simply of a pair of slightly modified longitudinal striæ.

The conoid neck becomes convex-conoid toward the truncated head, which is not set off in any way. There are six comparatively well amalgamated lips, each of which bears two innervated papillæ, one on the forward surface and somewhat forward pointing, and one on the outer surface and somewhat outward pointing. The anterior of these two papillæ is extended beyond the surface of the lip in the form of a minute seta or innervated papilla, and corresponds to the cephalic seta of other species of Diplogaster. The contour of the lip is not much disturbed by the presence of the posterior papilla, which is sometimes very difficult to see. Close behind the lateral papillæ or setæ there are minute openings in the cuticle, which in character closely simulate the amphids in some other species of Diplogaster, notably those of *D. fictor*. No doubt these are really the outward expression of minute amphids. Distally the lips have thin extensions which can close together over the pharynx in such a fashion that the front of the head is comparatively flat, though the tips of these lips may be recurved and point forward so as to make an exceedingly minute elevation at the middle of the front of the head. The latter has its front surface on the whole very slightly depressed.

The pharynx is about as deep as the front of the head is wide, and bears near its base on the dorsal side a relatively large, rather acute movable conoid tooth or onchus, which reaches about one-third the distance to the lips when the latter are closed, but which is relatively farther forward when the mouth is open. In addition there is a very much smaller submedian projection that undoubtedly may be denominated a rudimentary onchus. When the lips are closed the pharynx is a little wider at the base than anteriorly. At the base of the lips, opposite the posterior circlet of labial papillæ, the width of the pharynx is a little more than one-third that of the corresponding part of the head. Posteriorly, however, the width appears to be nearly three-fifths that of the corresponding portion of the head, at least when the head is viewed in profile. The walls of the pharynx are thin but refractive and fairly well ceratinized. The surface of the dorsal onchus is more highly ceratinized than that of other portions of the pharynx. Both the onchus and the wall of the pharynx have a yellowish or brownish color like that of the spicula. The end of the esophagus receives the base of the pharynx and is at once fully two-thirds as wide as the corresponding portion of the head. It continues to have the same diameter for some distance, then begins to expand and continues to do so to somewhat behind the middle of the neck, where it rather suddenly diminishes in diameter in such a way that it is proper to speak of a median bulb, although the anterior end of this bulb is not very distinctly set off by constriction from the anterior esophageal tube. This bulb contains an elongated valvular apparatus which is about one-third as wide as the bulb itself. This latter is three-fourths as wide as the corresponding portion of the neck. Notwithstanding the rather massive character of this median bulb, the succeeding portion of the esophagus is only about one-fourth as wide as the corresponding portion of the neck. However, it soon begins to widen and forms a somewhat pyriform cardiac bulb three-fourths as wide as the base of the neck. This bulb does not contain any very evident valvular apparatus, though in it there are faint indications of a modification of the esophageal lining. The intestine joins the posterior surface of the cardiac swelling, and at this point is about one-third as wide as the corresponding portion of the body. There is no very distinct cardia. The intestine widens out rather gradually and attains a width at least half as great as that of the body.

The tail end of the female begins to taper from some distance in front of the anus. This latter is slightly raised, especially its broader posterior lip. Behind the anus the tail diminishes somewhat more rapidly for a short distance and thereafter tapers regularly to the hairfine terminus. From the anus the rectum, which is about as long

as the anal body diameter, extends inward and forward. Nothing definite is known with regard to the lateral fields.

From the well-developed, slightly depressed vulva the vagina leads inward at right angles to the ventral surface halfway across the body, where it joins the two symmetrically placed uteri. The internal female organs are double and reflexed, and the ovaries, which are rather narrow and packed with small ova arranged irregularly, reach back to the vulva or even beyond. The ellipsoidal eggs are about as long as the body is wide and about two-thirds as wide as long. Their shells are smooth and rather thick. Specimens have been seen in which well-developed embryos existed in the eggs contained in the uteri. Other specimens have been found in which two to three dozen embryos had escaped from the eggs and then devoured the whole interior of the mother's body. The excretory pore is located opposite the cardiac swelling.

Male formula. $\frac{.9\ \ 11.\ \ 15.\ \ 'M^{68}.\ \ 89.}{2.2\ \ 5.4\ \ 6.1\ \ 10.\ \ 4.6}$ 0.8 mm. The tail of the male diminishes suddenly in diameter from the raised anus in such fashion that at a distance from the anus not very much greater than the anal body diameter it has a diameter only about one-fourth to one-fifth as great as at the anus. At this point, which is immediately behind the posterior group of male papillæ, the tail begins to taper rather gradually and somewhat uniformly, and continues so to do to the hairfine terminus, though there is at first a very slight increase in the diameter, so that the tail has the appearance of being very slightly constricted just behind the posterior caudal group of male papillæ. There is no spinneret, and there are no caudal glands. The two equal, rather slender, tapering, arcuate, brownish, acute spicula are about one and one-half times as long as the anal body diameter. At their widest part, a little distance behind the cephala, the spicula have a width about one-tenth as great as that of the corresponding portion of the body. From this widest part they taper gently toward the cephalated proximal ends. In the other direction the spicula taper regularly to their acute terminals. The accessory pieces surround the spicula at their distal extremities. The portion of the spiculum surrounded by the accessory piece constitutes about one-sixth of the length of the former. Extending backward from this encircling part of the accessory piece is a median arcuate portion arranged nearly parallel to the spicula and having its proximal end somewhat cephalated. The entire length of the accessory piece, including this median dorsal portion, is about one-third that of the spicula. Like the spicula the accessory pieces are brownish in color.

The hemispherical-conoid innervated supplementary male organs are located as follows: In front of the anus three pairs, two of which are ventrally submedian and one sublateral; the sublateral pair is nearly opposite the middle of the spicula, and is on nearly the same zone as the posterior of the two ventrally submedian pairs; the anterior submedian pair is a little in front of the proximal ends of the spicula. Behind the anus the papillæ are arranged as follows: One pair subventral or ventrally submedian immediately behind the anus, two pairs sublateral, and three closely approximated pairs of small size, subventral. This latter group of three pairs is slightly farther behind the anus than the foremost preanal pair is in front of it. The three pairs do not appear to be uniform in structure, the two anterior appearing to be mere innervations, while the posterior one is a distinctly raised innervated papilla like the preanal ones. The posterior of the two pairs of sublateral postanal papillæ is a trifle in front of the group of three just mentioned, while the anterior is about halfway between the group of three and the anus. The anterior border of the anus constitutes a sort of rudimentary flap with an innervation. The testis is single and rather broad and tubular. It extends forward and is reflexed a short distance behind the base of the neck. The reflexed narrower part of the testis is about twice as long as the corresponding body diameter.

Habitat: Manhattan, Kans. Found feeding on grasshopper eggs after the eggs had been deposited in the ground.

The eggs of *Diplogaster aerivora*, which are elliptical in shape, averaged about 0.062 mm. in length and 0.0335 mm. in diameter. When freshly deposited, they were dark brown in color, but became transparent as the embryo developed. Segmentation often began before the eggs were deposited and the succeeding cell divisions could (fig. 3, *F–M*) be readily followed throughout. The eggs were numerous and could be found lying close together in groups of from about 6 to 30. The eggs hatched in about 18 hours from the time segmentation was first noticed. Toward the end of the egg stage the living worm (fig. 3, *M*) could be plainly seen moving about within the egg wall. These movements became more active until the worm finally ruptured the wall and escaped.

At the time of hatching, the young nematodes (fig. 3, *B*) of this species averaged 0.2145 mm. in length. At this stage the sex organs could not be distinguished, because of their poor development. In water cultures the worms grew very rapidly and reached maturity in three to four days. The females matured slightly in advance of the males (fig. 2, *J*). *D. aerivora* never exceeded 0.5 mm. in length nor completed its life cycle while within the termite (fig. 3, *A*). The nematodes remained in the termite in this form for an indefinite length of time, but upon emerging into moist soil they matured in about two days.

Although molting occurred in this species as in *D. labiata*, it was much more difficult to observe; and, while it was not observed more than once in any individual, it is probable that more molts did occur. Molting required less time in *D. aerivora* than in *D. labiata*, and the posterior end of the nematode remained free throughout the process.

In the older water cultures the adults became so numerous that they appeared as a living mass to the naked eye. The females, which were much larger than the males, averaged 0.99 mm. in length and 0.067 mm. in diameter, while the males averaged 0.75 mm. in length and 0.046 mm. in diameter. When free in moist soil, the worms became even larger; the females (fig. 3, *D*) averaged 1.632 mm. in length and 0.1192 mm. in diameter, and the males (fig. 3, *E*) averaged 1.1425 mm. in length and 0.0724 mm. in diameter.

When reared in water cultures, the females appeared darker than the males, but when found in the soil both sexes appeared pearly white. The alimentary canal of the female, like that of *D. labiata*, was spiral, while that of the male was straight. The posterior end of the female's body tapered into a long, threadlike process, but in the male this process was shorter and its body ended in an abrupt hook.

PROCESS OF MATING.—The process of mating in *D. aerivora* (fig. 2, *L*) was much the same as in *D. labiata*. The male clasped the female slightly back of the middle of the body, so that its anal opening was in direct apposition to the genital pore of the female. In mating, the posterior end of the male usually completely circled the body of the female, although exceptions occurred. [illegible] many instances of mating

were observed, none lasted over 4½ minutes. As the mating neared completion, the female became more active and broke free.

RELATION AND ECONOMY OF THE SEXES.—Both males and females mated repeatedly with different individuals. A single female was observed to mate with 7 different males, and during this time laid a total of 317 fertile and 14 infertile eggs. The length of time from the first to the last mating was 13 days. The greatest number of fertile eggs produced from a single mating by any individual under observation was 125, but the average number was 52.63. A single male was successfully mated with 10 different females, the latter depositing 624 fertile eggs. The total time which elapsed during these 10 matings was 19 days.

TIME AND METHOD OF OVIPOSITION.—A single instance was observed of a female depositing a fertile egg 30 minutes after mating, although from one to two hours are usually required. The eggs developed in the ovaries in large numbers and were rapidly discharged through the genital pore. With age the females became very sluggish and did not appear to be able to discharge their eggs; consequently these eggs hatched within the body of their parent, where they fed on her internal organs. Usually they were unable to escape, although instances were observed where they escaped through the genital pore of the mother (fig. 2, *K*).

PROPORTION OF SEXES.—Three hundred specimens were examined, and of these 138 were males and 162 were females. In all cultures the females seemed to be more abundant.

HABITS.—These nematodes were found parasitic in the heads of *Leucotermes lucifugus*, where under natural conditions the number varied from 0 to about 75. Where heavy infestation occurred, the termites became sluggish and often died. These worms were usually more numerous in the immediate region of the mouth parts of *Leucotermes lucifugus*, although it was not uncommon to find them in the upper part of the cavity of the head. A great many termites were dissected, and in no case were nematodes found in the abdomen. In infested colonies nematodes were often seen in the surrounding soil. These usually were found in masses, feeding upon the bodies of dead termites or other available decaying matter. Specimens of *D. aerivora* placed in water cultures were found to flourish in the same food that was used for *D. labiata*. It was necessary to feed these nematodes each day, for without food they died in a very short time. As in *D. labiata*, the presence of food appeared to stimulate copulation and consequently caused an increase in oviposition.

So far as could be determined, these nematodes showed no preference to either day or night in mating, oviposition, or other habits.

LENGTH OF ACTIVE BREEDING STAGE.—The active breeding life of the female extended over a period of about 13 days, while that of the male was about 19 days. The complete life cycle of *D. aerivora* required from four to five days. As the individuals of this species which were

examined had no hibernation stage, their life cycle was continually repeated under favorable conditions. Insufficient moisture and lack of suitable food seriously interfered with the development of these nematodes.

A series of experiments was carried on to ascertain whether it is possible to introduce these parasites into *Leucotermes lucifugus*. Good cultures of nematodes were obtained in moist soil, into which specimens of *L. lucifugus* were placed. After two days a number of these termites were dissected, and it was found that there was an average of 22.9 nematodes in each head. In three days this average rose to 32.9 and in four days it was 46.6. In each instance the check count remained the same, being about 3 nematodes per head. After remaining in a similar culture for 12 days, all the termites died and the bodies were found to be literally alive with nematodes.

SUMMARY

(1) The eggs of *Diplogaster labiata* hatched in from 30 to 32 hours, while those of *D. aerivora* hatched in about 18 hours.

(2) The eggs of *D. labiata* were deposited singly, while those of *D. aerivora* were deposited in groups.

(3) More cases of eggs hatching in the body were found in *D. aerivora* than in *D. labiata*.

(4) The eggs of both species developed similarly.

(5) Both species, when reared in water cultures, used the same food, but in nature they had different hosts.

(6) Both species molted, but the process differed in that *D. labiata* fastened its posterior end, while *D. aerivora* did not.

(7) The adults of *D. aerivora* were larger than those of *D. labiata* and required much less time to mature.

(8) In water cultures, the females of both species were more numerous than the males.

(9) Although mating was similar in both species, *D. labiata* required more time for the process.

(10) Individuals of *D. labiata* usually mated but once, while those of *D. aerivora* mated repeatedly.

(11) Neither species in their habits showed any preference to day or night.

(12) The females of *D. aerivora* had a period of oviposition of about 13 days, while in *D. labiata* this period lasted only about 2 days.

(13) In both species adaptable and plentiful food acted as a stimulant to reproduction.

(14) Both species attacked insects, but in different regions of the body, as *D. aerivora* was found in the head while *D. labiata* was found in the intestines.

(15) The life cycle of *D. labiata* required more than twice as much time as did that of *D. aerivora*.

(16) *D. aerivora* was successfully introduced into the termites.

INSECT INJURY TO COTTON SEEDLINGS[1]

By B. R. COAD and R. W. HOWE, *Entomological Assistants, Southern Field Crop Insect Investigations, Bureau of Entomology*

INTRODUCTION

The present work deals with leaf mutilation of cotton seedlings (*Gossypium* spp.) caused by insects. The observations were made in the vicinity of Tallulah, La., during the spring of 1915. Such injury to cotton seedlings is probably found throughout the entire area of cotton cultivation in the United States. The senior author has noted it in many parts of Texas, both the drier and more humid portions, in Louisiana, and in Arizona on irrigated cotton. Since these localities approximate the extremes of rainfall, temperature, and sunshine under which cotton is cultivated, it is reasonable to expect the injury at almost any place.

CHARACTER OF INJURY

The injury varies much in appearance and intensity, but all of the examples which have come to the attention of the authors have certain more or less constant characteristics. This is frequently noticed as soon as the seedlings appear above the ground, although it may not appear until later. The time of the cessation is also variable, but it does not seem to continue after the plants reach a height of 10 to 12 inches and usually stops much earlier. In the vicinity of Tallulah this injury is seen from the first sprouting of the plants until the latter part of May.

The first appearance is characterized by irregular holes appearing in the cotyledons. These vary from small holes through the leaf or small marginal incisions to almost complete loss of the leaf. Following this the later leaves are attacked in the same manner, with all possible variations in the type and degree of the injury. In some cases the terminal bud may be lost.

LABORATORY STUDIES

Efforts were made to secure growing plants at the earliest possible date. For this purpose cotton seed was planted in boxes and pots in the laboratory during the very early spring, but lighting facilities were so poor at this season that the plants failed to thrive. The first healthy seedlings which were secured sprouted in the laboratory hotbed March 16 from seed planted in the middle of February. Seed planted in another part of this hotbed on March 5 sprouted well a little later. This hotbed

[1] The investigations upon which this paper is based were conducted under the direction of Mr. W. D. Hunter, in Charge of Southern Field Crop Insect Investigations, Bureau of Entomology.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
de

Vol. VI, No. 3
Apr. 17, 1916
K—29

was covered with glass during the night and was only opened during the warmer part of the day. The plants appeared perfectly healthy at all times and grew well.

Other plantings were made in the laboratory yard at intervals during March for studies under outside conditions. Later, seeds were germinated between layers of moist absorbent cotton and placed in pots containing soil sterilized by baking. These pots were then placed in large screen cages and the plants were allowed to grow under this protection.

The first injury was noted in the hotbed on March 31. These seedlings had sprouted March 16 and at this time were about 3 inches tall. They had been protected from cold by the glass covers, and the soil had been well manured. On this first morning a number of plants were found to have been injured.

Following this the progress of the injury was noted carefully. All plants were examined daily and those showing injury were tagged. In this manner a record of the number of plants injured each day was secured. On the morning of April 14 nine new seedlings were injured; on April 15 five, on April 16 six, on April 17 two, and on April 18 three.

In order to determine the period in which the injury was incurred, both morning and evening counts were started. These showed the number of seedlings injured during the night and during the day. These observations were started April 22 and continued until May 4. The results are presented in Table I.

TABLE I.—*Comparison of day and night injury to cotton seedlings*

Date of examination.	Number of seedlings injured during day.	Number of seedlings injured during night.	Date of examination.	Number of seedlings injured during day.	Number of seedlings injured during night.
Apr. 22	.....	5	May 1	0	1
23	7	6	2	0	3
26	4	10	3	0	4
27	2	2	4	0	3
28	4	5			
29	4	4	Total	26	49
30	5	6			

From this table it is seen that 66 per cent of the injury appeared during the night and 34 per cent during the day.

On April 14 this same type of injury appeared upon seedlings which had just sprouted in the laboratory garden, and from that time it appeared about as abundantly here as in the hotbed.

The rapidity with which the injury was produced was quite striking, and special studies were made upon this point. A number of apparently healthy and entire seedlings were examined morning and evening, and in that way the amount of injury produced in a single night was

determined. This was done in both the hotbed and the garden, and the results were the same in both cases. Leaves which were entire and uninjured at nightfall would show large holes often occupying one-half of their area on the following morning. Later observations have shown practically entire leaves disappearing in the same manner during the night.

During the first few days when the injury was appearing in the hotbed a number of examinations were made during the daytime in the attempt to find some insect producing the injury, but not a single individual which could be suspected of being the cause was noted. However, on April 6, 50 square inches of the hotbed soil were examined to a depth of 3 inches, and 12 cutworms were found. If this was a fair sample of the hotbed, the soil there certainly contained hundreds of the worms. Eleven of these larvæ were very small, while one was about an inch in length.

The presence of these larvæ in the hotbed and the fact that they were known to feed upon plant leaves made it seem quite possible that they were responsible for more or less of the injury. Consequently several examinations were made at night, and a number of cutworms were found feeding on the leaves of the plants. At this time the same injury was noted on clover and weed leaves in the hotbed.

Several half-grown cutworm larvæ collected on cotton in the garden and hotbed were placed on the surface of the soil in a pot containing a number of seedlings. This pot was placed in a screen cage and the larvæ attacked the seedlings at once. Plate XII, and Plate XIII, figure 1, show several seedlings injured by these larvæ.

STUDIES OF CLIMATIC FACTORS

A number of tests were conducted to determine whether any of the injury could be due to the exposure to low temperatures during the night or to the hot sunlight in the morning before the plants had time to become warm. In the first test a wooden frame was erected over a cotton planting in the laboratory garden just prior to the sprouting of the plants. This frame was 2½ feet in height and was covered with 8-ounce duck. This cloth was placed over the frame at sundown each day and allowed to remain until about 10 o'clock the following morning. In this manner the radiation was reduced under this cover during the night and the plants were protected from sudden exposure to the sunlight in the early morning. A minimum thermometer was suspended under the cover in the center of the bed about 15 inches from the ground and another was suspended at the same height in the open garden a few feet away. Records continued for a few nights showed only a slightly higher temperature under the shelter, so the frame was lowered to within 1½ feet of the ground and the thermometers were lowered to 6 inches. Following this the minimum temperatures under the cover usually ranged a few degrees higher than in the open.

This frame was first erected April 26 and on April 30 the first seedlings appeared above the ground. Of the 18 which sprouted this first day, 5 showed injury. On May 1, 9 of the 45 seedlings showing above the ground were injured, while on May 3, 10 out of 50 were injured. On May 4, 16 out of 70 and on May 5, 22 out of 70 were injured. These observations were continued until May 8 and new seedlings were injured practically every day.

On May 8 a second test of the same sort was started. In this case, however, the cotton row was covered just before sprouting with heavy pasteboard boxes, 1 foot square and 8 feet long. These boxes were covered with several layers of 8-ounce duck and were only removed from over the plants during the hotter part of the day. Minimum thermometers were arranged under the boxes and in the open in the same manner as that just described in the preceding test. In this case considerable differences in the nightly minimum temperatures were noted. It was usually from 3 to 6 degrees warmer under the box than in the open. On May 12 the first seedlings appeared, and of the 39 in sight, 3 showed injury to the leaves. This test was continued six days longer and the injury continued to appear.

For comparison with the seedlings growing in the garden and hotbed, a number of seeds were planted at intervals in pots and crocks containing soil sterilized by baking. Part of these were allowed to remain exposed in the open, while others were placed in screen cages. In the hundred or more seedlings grown in this manner not a single sign of injury was found, whereas the injury was appearing abundantly on plants growing in the garden and hotbed at this same time. From this it seemed quite evident that the cause of the injury was located in the soil which had not been baked.

FIELD OBSERVATIONS

As the injury was appearing in the various fields at this same time, efforts were made to learn its extent and to discover any insects which might cause the lesions. In these studies all insects which were known to be leaf feeders were noted and an attempt was made to secure positive samples of their injury to cotton. On April 19 four small lepidopterous larvæ were found feeding upon the leaves of cotton seedlings at a plantation near Tallulah. The injury which they were producing was apparently identical with that already noted. These larvæ belong to the family Liparidae and are commonly known as "tussock moths" (*Hemerocampa leucostigma* Smith and Abbot). On this same date three larvæ of the same species were found feeding on the seedlings in the hotbed and one was found in the laboratory garden. Following this the field examinations showed a considerable number of these larvæ to be present around Tallulah, and associated with them were found several species of cutworms and "measuring worms." All produced nearly the same type of injury to the seedlings.

In order to determine definitely the amount of injury present in the various cotton fields around Tallulah and also the prevalence of the worms, a considerable number of examinations were made during the latter part of April. In these observations only the worms found on the cotton seedlings were noted. In order to make the figures more accurately represent the condition of the field, the plants were examined in groups of 100 each in all parts of the field. The results are summarized in Table II.

TABLE II.—*Records of examinations for insect injury to cotton seedlings in fields around Tallulah, La.*

Date.	Number of seedlings examined.	Number of seedlings injured.	Per cent of seedlings injured.	Number of lepidopterous larvæ found.	Type of soil.	Remarks.
Apr. 20	1,000	266	26.6	25	Sandy	All tussock larvæ; very small.
21	200	30	15.0	.....	...do	Seedlings just above the ground.
22 and 23	2,300	534	32.2	25	...do	Eleven cutworms and 14 tussock larvæ.
22	1,000	84	8.4	1	Buckshot	Cutworm.
22	800	188	23.5	1	Sandy	Do.
22	800	54	6.7	.....	Buckshot	
24	2,300	207	13.4	9	Sandy	Six cutworms and 3 tussock larvæ.
27	1,200	380	31.7	4	...do	Two geometrid larvæ and 2 tussock larvæ.
27	1,000	227	22.7	1	...do	Tussock larva.
29	400	43	10.7	.....	...do	
Total	11,000	2,013	.....	66	.....	Forty-five tussock larvæ, 19 cutworms, and 2 geometrids.
Weighted average	.....	.....	18.3	.....	.....	

From this it is seen that the percentage of plants injured at the various plantations visited ranged from 6.7 to 32, with an average of 18.3 per cent for the 11,000 seedlings examined. In the course of these observations 66 lepidopterous larvæ in all were found. By far the greater part of these were the "tussock" larvæ and the remainder were either cutworms or "measuring worms."

The possibility of the soils having some influence upon the extent of damage was considered, but the writers were unable to secure sufficient information to allow definite conclusions. Soils in the vicinity of Tallulah may be roughly classed as either "sandy" or "buckshot." The former is the light, sandy land found on the bayou fronts, while the "buckshot" is the dark, heavy, stiff "back land." Under boll-weevil conditions "buckshot" land is not adapted to cotton culture; hence, only two fields of this type of soil were located for study. The percentage of injured seedlings in these two fields was 6.7 and 8.4. These were the lowest records made and are considerably below the average of sandy fields near by. Whether or not this lesser degree of injury was due to the soil is open to doubt. Owing to the "coldness" of "buckshot" land in the spring, the cottonseed germinates slowly and consequently the plants were considerably smaller

than those on sandy land. This may have caused the difference in the percentage of injury. However, only one suspected larva (a cutworm) was found in the two fields.

The different lepidopterous larvæ noted were all observed to be feeding upon the leaves. The tussock larvæ were much the more abundant and evidently produced a great deal of the injury. During the earlier examinations nearly all of these tussock larvæ were quite small. The injury produced varied somewhat with the size of the larva. The very small individuals fed only upon the epithelium of the lower side of the leaf and the injury was not visible from above. With a slight increase in size the larvæ started to feed through the leaf and at this stage produced the peculiar type of injury shown in Plate XIII, figure 2. Later the older larvæ (one-half to full grown) ate large holes in the leaves, and the injury could no longer be distinguished from that of the other species concerned. Plate XIII, figure 3, shows the injury produced by one nearly full-grown tussock larva when confined in a large screen cage with cotton seedlings growing in a pot.

About May 1 nearly all cotton fields under observation suddenly began to show greatly increased injury until within a few days many fields had practically every plant more or less mutilated. This proved to be due to an invasion of grasshopper nymphs. These speedily became very abundant and swarmed over the young cotton, feeding principally upon the leaves. This is shown in Plates XIV and XV. These cotton leaves were collected in the field when the young grasshoppers were feeding upon them.

A little later in May the 12-spotted cucumber beetle, or adult of the southern corn rootworm (*Diabrotica 12-punctata* Olivier), became abundant locally and added to the injury. The work of these beetles closely resembled that of the worms and grasshoppers, though the holes made were usually not very large. At this same time woolly-bear larvæ began to appear in the fields and produced the same injury.

Following this great increase in injury to the plants caused by the grasshoppers, counts were made to determine the percentage of injured seedlings in four average fields near Tallulah. The information secured from these examinations is shown in Table III.

TABLE III.—*Abundance of injured cotton seedlings after the grasshopper invasion*

Date.	Number of seedlings examined.	Number of seedlings injured.	Percentage injured.
May 14	800	792	99.0
15	3,500	3,446	98.5
17	2,000	1,920	96.0
17	1,000	1,000	100.0
Total	7,300	7,158	.....
Weighted average	.....	.....	98.0

Here it is seen that 98 per cent of the 7,300 seedlings examined had been injured by some of the various agencies operating prior to that time. High as they are, these figures are representative of average conditions in the fields near Tallulah.

ACTIVE PERIOD OF LARVÆ

On April 14 continuous examinations of cotton seedlings were made from 8 a. m. until noon and from 1 to 5 p. m. on two plantations near Tallulah. The day's records of worm collections were divided into hourly periods and in this manner the active time of the various larvæ was noted. The results of these studies are shown in Table IV. From this it is seen that the tussock larvæ were much the more abundant throughout the day and there seemed to be no time at which they were especially abundant on the plants. The same seems to be true of the other larvæ.

TABLE IV.—*Records of field examinations for larvæ by hourly periods on two plantations near Tallulah, La.*

Period.	Number and kinds of larvæ found.	
	First plantation.	Second plantation.
8 a. m. to 9 a. m.	2 tussock larvæ, 1 cutworm.	1 tussock larva.
9 a. m. to 10 a. m.	7 tussock larvæ.	4 cutworms, 2 yellow "woolly-bear" larvæ.
10 a. m. to 11 a. m.	9 tussock larvæ.	9 tussock larvæ, 2 cutworms, 3 yellow "woolly-bear" larvæ.
11 a. m. to 12 noon	3 tussock larvæ, 2 small cutworms.	No examinations.
1 p. m. to 2 p. m.	7 tussock larvæ, 1 small cutworm, 1 yellow "woolly-bear" larva.	No worms.
2 p. m. to 3 p. m.	6 tussock larvæ.	2 unknown larvæ.
3 p. m. to 4 p. m.	6 tussock larvæ, 1 small cutworm.	3 unknown larvæ.
4 p. m. to 5 p. m.	7 tussock larvæ, 2 yellow "woolly-bear" larvæ.	1 tussock larva, 1 geometrid, 6 unknown larvæ.
Summary.	57 tussock larvæ, 5 cutworms, and 3 yellow "woolly-bear" larvæ.	14 tussock larvæ, 8 cutworms, 5 yellow "woolly bears," 1 geometrid, and 11 unknown larvæ.
Total, both plantations.	71 tussock larvæ, 13 cutworms, 8 yellow "woolly-bear" larvæ, 1 geometrid, and 11 unknown larvæ.	

INJURY TO TERMINAL BUDS

The greater part of the feeding of the insects just mentioned is confined to the leaves. However, a considerable number of plants were found with the terminal buds either partially or completely destroyed. Plate XVI, figure 1, shows the usual location of this injury. This seedling was found in the field with a lepidopterous larva embedded at the base of the bud (*a*). The small cavity where the larva was feeding is shown in the photograph. From this the injury progresses until often all the buds and small leaves above point *a* are eaten out.

ULTIMATE EFFECT OF INJURY UPON THE PLANTS

The preceding pages have shown the different insects contributing to the mutilation of cotton seedlings, but it is the ultimate effect upon the cotton production of the plants which determines the economic importance of the injury. This is a point upon which it is difficult to secure accurate data, but a certain amount of information has been gathered by the writers.

A number of plants are evidently killed outright by the feeding of the insects; but this number appears to be so small, even in fields very heavily infested, that it is of no practical importance.

The leaf feeding is also of very doubtful importance. In severe cases it retards the growth of the plants somewhat and occasionally dwarfs them permanently, but usually they recover very rapidly, and there is no visible effect other than the slight retardation.

Apparently it is the injury to the terminal buds which produces the most important economic effect. When this bud is injured or destroyed, the development of the plant is greatly changed. Instead of having a single main stem extending to the top of the plant, two or more large branches develop just below the injured bud and serve as stalks to produce the fruiting branches. Usually several very abnormal clusters of leaves form around the stalk near the injury. In Plate XVI, figure 2, the result of similar injury is shown in comparison with a normal plant. These two plants were collected in the garden at the laboratory and were stripped of their leaves before being photographed. Plant *B* shows a normally developed stalk and its branches, while plant *A* shows the deformity caused by the destruction of the terminal bud.

About the middle of June a number of examinations were made in the fields near Tallulah in order to determine the abundance of these deformed plants. The results of these examinations are given in Table V.

TABLE V.—*Records of field examinations for deformed cotton plants at Tallulah, La.*

Date.	Number of plants examined.	Number of plants deformed.	Percentage deformed.	Location.
June 8	4,000	314	7.8	Plantation.
9	100	3	3.0	Hotbed.[1]
9	100	7	7.0	Laboratory garden.[1]
10	1,000	87	8.7	Plantation.
11	100	4	4.0	Do.
11	500	63	12.6	Do.
16	400	33	8.2	Do.
17	600	42	7.0	Do.
Total	6,800	553	.....	
Weighted average	.....	.....	8.1	

[1] Just prior to this examination the plants in the garden and hotbed had been hand thinned; and as the poorest plants were removed, the percentage of deformed plants was evidently greatly lowered.

From this it is seen that the percentage of deformed plants ranged from 3 to 10.6, with an average of 8.1. As these same fields furnished the records given in Tables II and III, and were shown in the latter to have practically every plant more or less mutilated, it seems evident that only a comparatively small amount of the injury produces final deformity. However, an injury which deforms only 8 per cent of the plants in a field still is of considerable importance.

When this deformity was first observed it was at once noted that the injured plants were not forming as many squares as normal plants of the same age and height. Further studies showed this effect to be so pronounced that counts were made in the fields to determine the relative squaring of deformed and normal plants. In these observations, every time a deformed plant was found its squares were counted, and likewise those on the nearest eight normal plants of the same size. The average of these normal plants was compared with the number upon the deformed one. In 40 cases out of the 229 recorded the squares on the injured plants exceeded the average of the nearby normal plants, but in all others the average of the normal ones was considerably higher than the number on the injured plants. A summary of these observations is given in Table VI.

TABLE VI.—*Effect of deformity upon fruiting of cotton plants*

Date.	Deformed plants.				Normal plants.			
	Number observed.	Total squares.	Average squares per plant.	Maximum squares per plant.	Number observed.	Total squares.	Average squares per plant.	Maximum squares per plant.
June 10.....	87	248	2.8	10	700	3,804	5.4	16
11.....	4	23	5.0	9	32	267	8.3	13
11.....	63	52	0.8	6	502	1,105	2.2	10
16.....	33	405	12.3	26	264	3,931	14.9	34
17.....	42	559	13.1	34	336	6,122	18.2	53
Total.....	229	1,287	.....	.....	1,834	15,229	.....	.....
Weighted averages	.....	.....	5.6	.....	.....	.....	8.2	.....

The 229 deformed plants averaged 5.6 squares per plant, while the 1,834 normal ones averaged 8.2 squares. This gives a difference of 2.6 squares per plant in favor of the normal plants at the time of these observations.

From these figures it is evident that the necessity for the additional vegetative development before squaring retards the fruiting of the plants considerably. This is a point of great importance in cotton culture under boll-weevil conditions. The primary requisite for a successful crop in the presence of the boll weevil is early, rapid, and prolific fruiting. This allows the safe "setting" of a crop before the weevils multiply

sufficiently to infest all the squares. Hence, any agency which retards the formation of the squares in the early spring does a very serious injury to the crop. While the deformed plants may overtake the normal plants later in the quantity of fruit, this fruit will be produced too late to insure safe maturing.

Another effect of the deformity which may be of considerable importance is the ease with which the plants are split when the two or more branches fork at the same point. This gives a very weak stalk, and a comparatively slight jar will split it. In fact, the weight of a crop of bolls will break many of the plants.

SUMMARY AND CONCLUSIONS

From the various observations discussed in this paper it seems that mutilation of cotton seedlings may be produced by any of several insect pests. These consist of a number of species of lepidopterous larvæ (cutworms, measuring worms, "woolly-bear" larvæ, tussock-moth larvæ, etc.), grasshoppers, and leaf beetles. In all fields several species of these pests were present, and in many fields all of them were found. During the spring of 1915 at Tallulah, La., the tussock larvæ were responsible for most of the damage early in the season and then were supplanted by the grasshopper nymphs. However, the relative importance of the various species undoubtedly varies with the locality and season.

Tests made with plants protected from low temperatures during the night and from bright sunshine in the early morning demonstrated that the injury would appear about as abundantly on these plants as on the unsheltered plants in the garden and field. Seedlings in large number, raised through this period in pots and crocks containing baked soil, failed to show the slightest trace of injury, although they were fully exposed to the weather.

Injury to cotton by cutworms has been known for many years, but usually has been considered to consist only of the cutting of the plant stem near the ground. In 1897 Howard [1] published a brief review of the information then available concerning these larvæ, but did not mention them as leaf feeders. In 1905 Sanderson [2] mentioned the injury due to these worms and also discussed the work of *Prodenia ornithogalli*. This species he recorded as being diurnal in habits and feeding upon the leaves, but he considered the damage to the squares and bolls as its most important injury. Sanderson also mentioned the "woolly-bears" as occasionally damaging cotton by feeding upon the leaves.

In actual effect upon the plants it seems that the injury of the various species may result in death of the plant, dwarfing of growth, or deformity

[1] Howard, L. O. Insects affecting the cotton plant. U. S. Dept. Agr. Farmers' Bul. 47, 32 p., 18 fig. 1897.

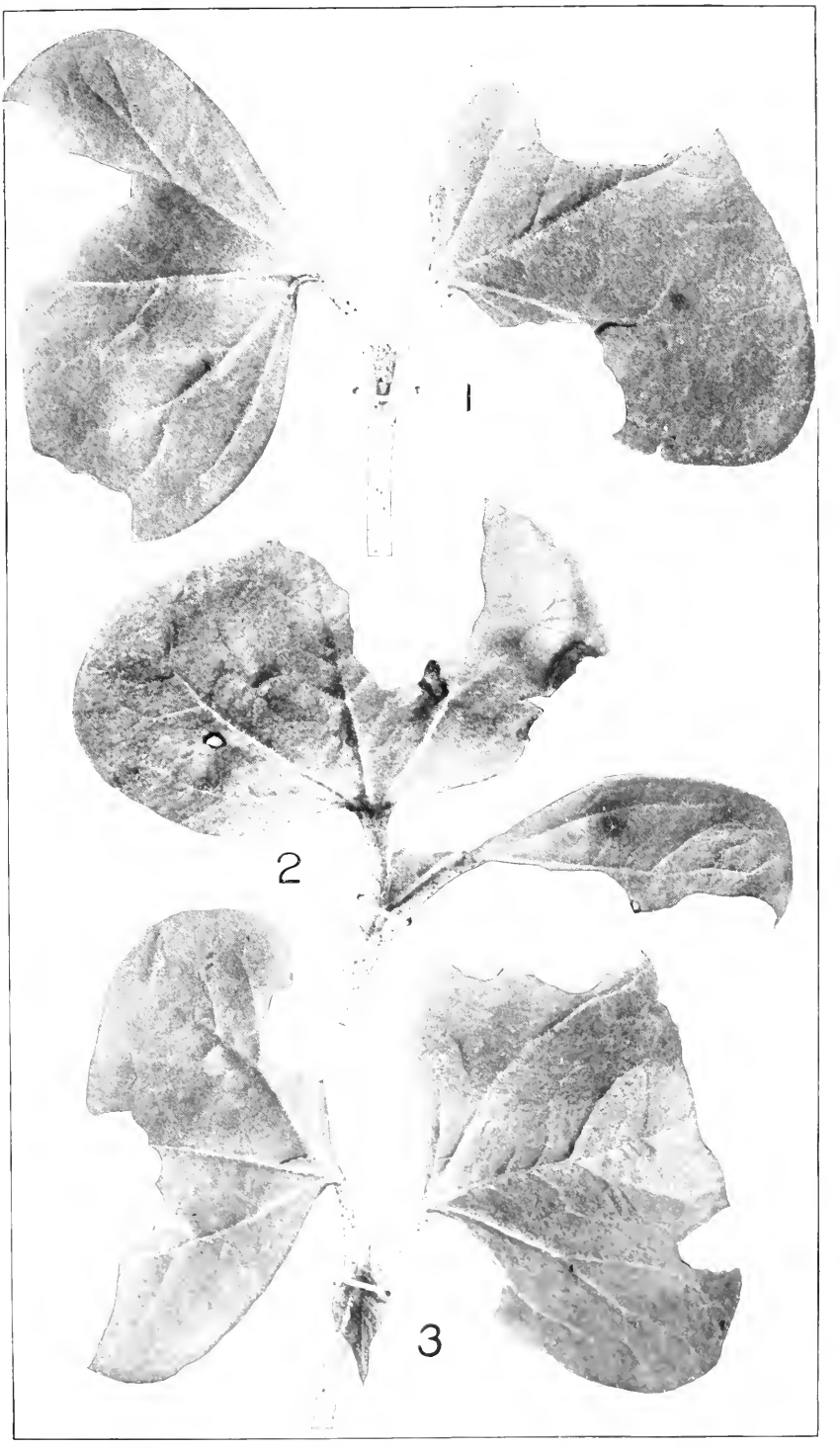
[2] Sanderson, E. D. Miscellaneous cotton insects in Texas. U. S. Dept. Agr. Farmers' Bul. 223, 24 p., 29 fig. 1905.

of the stem, producing retardation of the fruiting. Of these the deforming of the stalk is evidently much the more important. Field examinations have shown that an average of 8 per cent of the plants in the fields under observation were deformed and that these abnormal plants averaged 2.6 squares per plant less than the normal ones about the middle of June. As the cotton in these fields averages about 4 feet between the rows and is spaced about 18 inches in the drill, this would mean a loss of over 1,500 squares per acre at the critical period in cotton production in the presence of boll weevils.

The "woolly-bear" larvæ mentioned in this paper were reared and proved to be *Estigmene acraea* Drury. Two of the cutworms have been identified by Mr. S. E. Crumb, of the Bureau of Entomology, as *Prodenia ornithogalli* Guenée and *Peridroma margaritosa* Haworth, var. *saucia* Hübner.

PLATE XII

Fig. 1.—Cutworm injury to cotton seedlings; produced in breeding cages.
Fig. 2, 3.—Cutworm injury to cotton seedling.


1
2
3

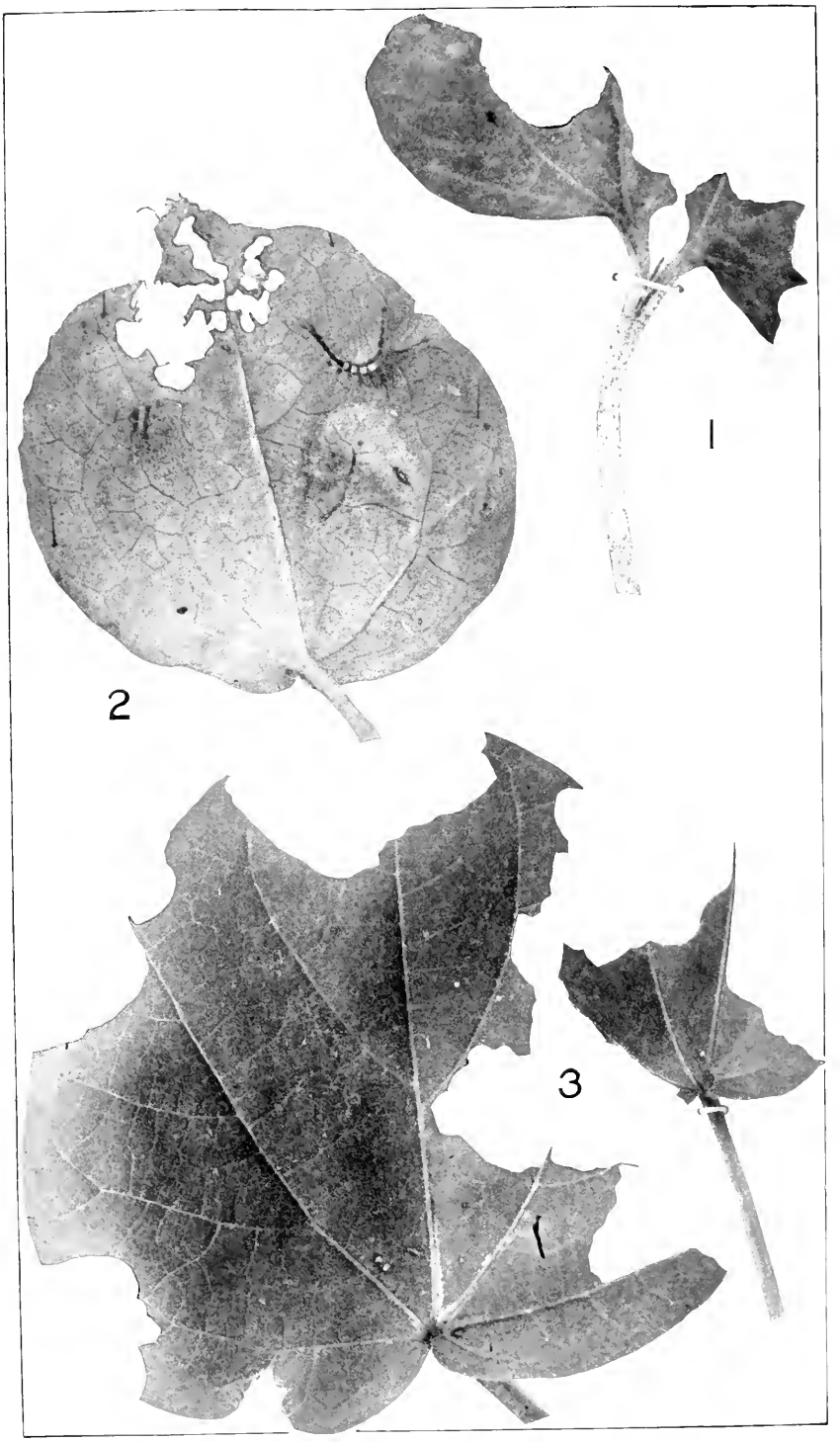

1
2
3

PLATE XIII

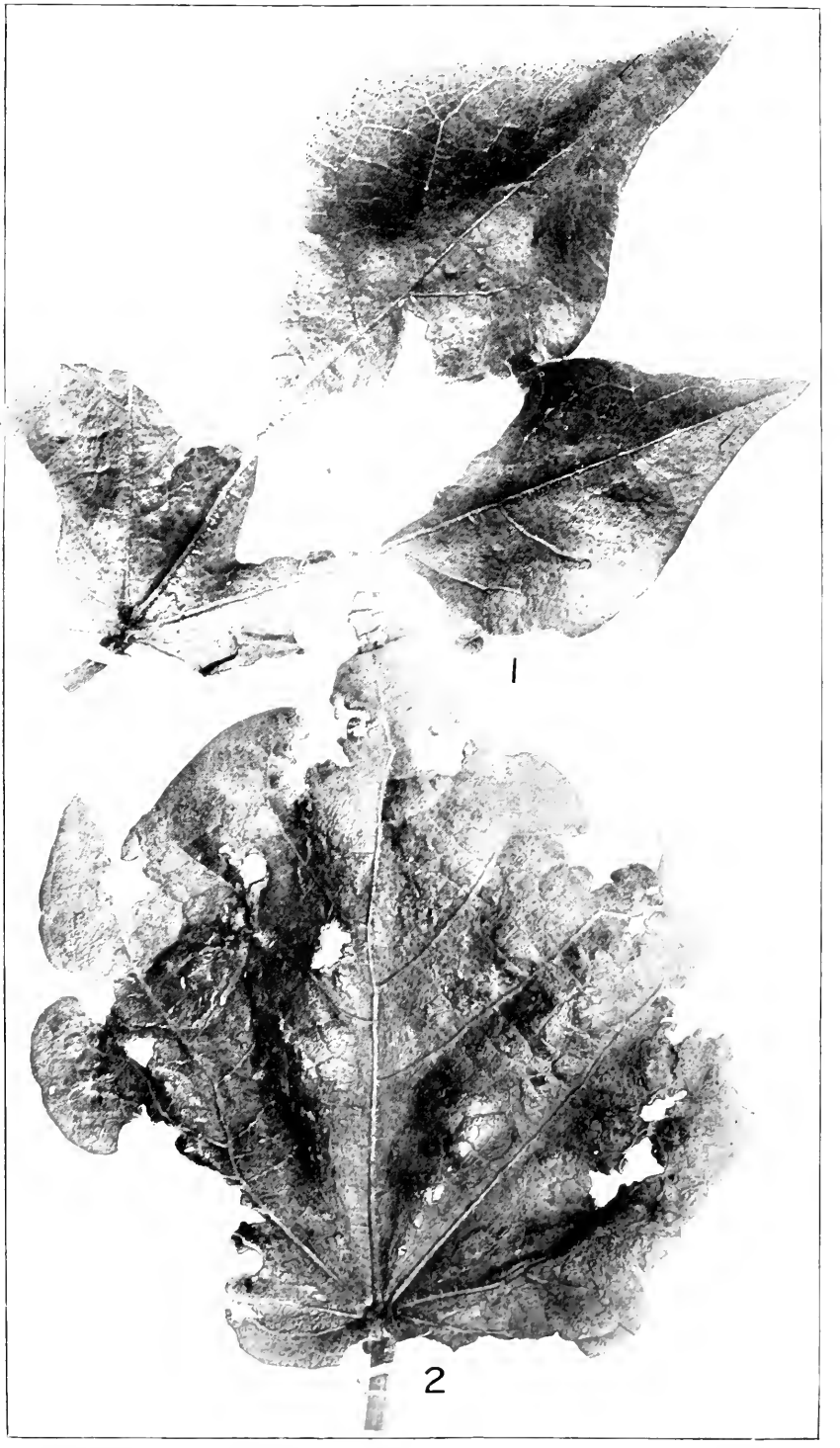
Fig. 1.—Cutworm injury to cotton seedling.

Fig. 2.—Tussock larva feeding upon cotton leaf. The ragged injury shown here is usually produced by the smaller larvæ.

Fig. 3.—Injury produced by a nearly full-grown tussock larva when confined in a screen cage containing potted cotton plants.

PLATE XIV

Cotton leaves showing grasshopper injury.


1
2


1
2

PLATE XV

Fig. 1.—Underside of cotton leaf showing grasshopper injury. This shows a number of places where the very small nymphs ate only the epithelium and did not penetrate the leaf.

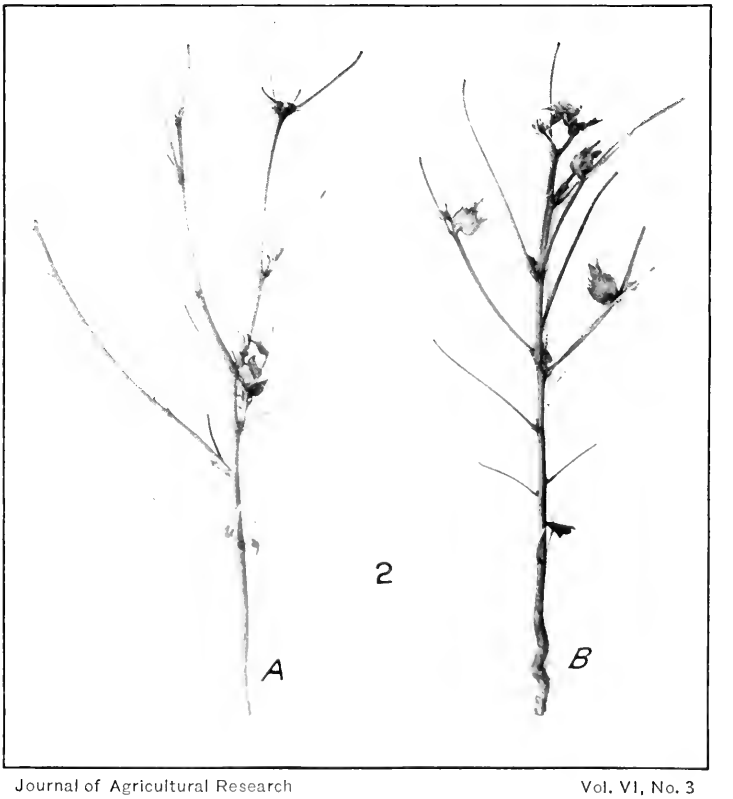
Fig. 2.—Cotton leaf showing grasshopper injury.

PLATE XVI

Fig. 1.—Injury to terminal bud of cotton by lepidopterous larva. This worm was embedded at point *a*.

Fig. 2.—Two cotton plants from laboratory garden with leaves removed. Plant *A* shows the abnormal forking caused by injury to the terminal bud, while *B* is a normal stalk. The absence of fruit on plant *A* is due to the deformity.


a
1


2
A
B

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

15 CENTS PER COPY
SUBSCRIPTION PRICE, $3 PER YEAR

▽

Vol. VI APRIL 24, 1916 No. 4

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

EDITORIAL COMMITTEE

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., APRIL 24, 1916 No. 4

A SEX-LIMITED COLOR IN AYRSHIRE CATTLE[1]

By EDWARD N. WENTWORTH,
Professor of Animal Breeding, Kansas Agricultural Experiment Station

TYPES OF INHERITANCE AS RELATED TO SEX

Two general types of inheritance as related to sex exist, aside from the ordinary secondary sex characters. Sex-linked inheritance depends on the great mass of hereditary factors that have been shown to be linked in transmission to the sex-determining factors; while sex-limited factors follow the simple Mendelian scheme of inheritance, but show a reversal of dominance in the two sexes. Frequently these two latter terms are used synonymously, but since there is a distinction between the two classes of transmission, and since the term "sex linked" is so much more descriptive of the hereditary phenomena to which it has been applied than is the term "sex limited," the foregoing terminology is used.

HISTORICAL REVIEW

The classical case of sex-limited inheritance was reported by Wood (7), who made reciprocal crosses of the Dorset sheep, a breed horned in both sexes, with the Suffolk, a breed polled in both sexes. All F_1 individuals were the same, so far as the type of cross was concerned, the males being horned and the females polled. In the F_2 generation the fact that dominance differed in the two sexes resulted in three males being horned to one being polled, and three females being polled to one being horned.

Similarly in 1912 the writer reported a pair of rudimentary teats in swine, located on the lower part of the scrotum of the male and on the inner thighs of the female, behind the inguinal pair, which presented the same phenomenon in transmission, the character being dominant in the male and recessive in the female.

Gerould (2)[2] reported in 1911 on the inheritance of yellow and white in the common clover butterfly (*Colias philodice*). White is dominant to

[1] Paper No. 3 from the Laboratory of Animal Technology, Kansas Agricultural Experiment Station.
[2] Reference is made by number to "Literature cited," p. 147.

yellow in the female, but it is recessive in the male. Something lethal seems to be connected with homozygosis for white; hence, white as a somatic character appears only in the female. The yellow female is YY, the white female YW. Males are either YY or YW, but are always yellow.

Jacobson (3) made some observations on *Papilio mennon* L., which were studied from a Mendelian standpoint by De Meijere (5) in 1910. There are three varieties of females in this species known as Achates, Agenor, and Laomedon, respectively, in the order of their dominance. The males corresponding to these three forms are all alike, although each of the female patterns may be carried in a recessive manner. Furthermore, De Meijere believes that the female carries the male pattern homozygously; but, owing to the reversal of dominance, the male character never becomes somatic. The Laomedon probably represents the female expression of the male condition. The principal difference between this and the previous cases is that the changes in dominance affect the homozygotes as well as the heterozygotes.

AYRSHIRE BLACK-AND-WHITE

A case which seems to fall under this general sex-limited group is found in the inheritance of black-and-white as alternative to red-and-white in Ayrshire cattle. While the general breed color is red-and-white, black-and-white animals have been known for some time, as shown by Kuhlman (4). Practically no attention has been paid to the mode of inheritance of this color, since in America it has been considered undesirable and selection against it has been practiced. It is difficult to state whether the black is due to a true black pigment or whether it is simply a very dense red. Under the microscope typically black granules seem to be present, but no chemical solutions of the pigments have yet been attempted.

SOURCE OF THE DATA [1]

The Ayrshire herd bull at the Kansas Experiment Station, Melrose Good Gift, is a very deep mahogany-and-white; in fact, the black-and-white previously referred to. It is through the study of his ancestry and breeding performance, the ancestry and breeding performances of the cows in the herd, including the black-and-white animals, and the records of some of the former herd bulls that the present data were secured. In all, 63 individuals were included. Much larger numbers might have been obtained by adding the progeny of red-and-white males and females to the table; but since they demonstrated no facts different from those here included, their records are not presented.

[1] Acknowledgments are hereby made to Prof. O. E. Reed, of the Department of Dairy Husbandry, Kansas Experiment Station, for facilities extended in obtaining the data.

PROGENY OF MELROSE GOOD GIFT FROM RED-AND-WHITE COWS

Fifteen red-and-white cows in the herd were mated to Melrose Good Gift to produce 20 calves, of which 10 were black-and-white bulls and 10 were red-and-white heifers. All of the bulls were as red as the heifers at birth, but at 2 to 4 months of age the blackish tinge began to develop, and within 4 months the youngsters became distinctly black-and-white. The heterozygous male progeny of Melrose Good Gift differed from the homozygous male progeny in that the black tinge developed more slowly and also became much less intense on maturity. While in the mature homozygous bull the black is very distinct throughout the pigmented areas, in the mature heterozygous bull the black may appear only as a streaked border where the pigmented spots adjoin the white, or at the limbs, muzzle, ears, and tail. The main portions of the colored parts of the animal are usually a very dark red which blends gradually, although in a particulate manner, into the blacker borders. The heterozygous heifers are red-and-white, and while occasional dark hairs are found, no regular means whereby the heterozygous red-and-white females could be distinguished from the homozygous red-and-white females was discovered. It should be further noted that the black color of the homozygous female is by no means as intense as that of the male, although the black is indisputably present.

HETEROZYGOUS BLACK BULLS TO HOMOZYGOUS RED COWS

Johanna Croft King, College Marquis, Sir Croft of Spring City, Woolford's Good Gift, and Lessnessock Oyama's Good Gift were bulls which by their breeding performance and somatic description must have been heterozygous for the black factor. The last two bulls were found in the pedigree of Melrose Good Gift, while the first three were used at one time or another at the college as herd bulls. Records of these in matings to homozygous red-and-white cows were available for all except Woolford's Good Gift, and the result showed four red-and-white heifers, four black-and-white bulls, and 5 red-and-white bulls. This is the most probable distribution of colors in both the males and females and is perfectly in alignment with the interpretation of the method of inheritance as given.

The reciprocal cross of red-and-white bulls to black-and-white cows gave two black bulls to one red bull and two white heifers, also the most probable expectation.

BLACK-AND-WHITE COWS MATED TO RED-AND-WHITE BULLS

Only three calves were available from this type of mating, all red-and-white daughters of Bangora, the original black-and-white cow in the herd. While the numbers are too small to be conclusive, yet they conform to the expectation.

RESULTS OF THE DIFFERENT CROSSES

If the factor for the black-and-white color is represented by B, the hereditary constitutions are as follows: BB is always black-and-white; bb is always red-and-white; Bb is always black-and-white in the male and red-and-white in the female. All of the nine possible matings were discovered, as shown in Table I.

TABLE I.—*Results of nine possible matings of Ayrshire cattle*

Sires.	Dams.	Male offspring.		Female offspring.	
		Black-and-white.	Red-and-white.	Black-and-white.	Red-and-white.
BB	BB	1	0	3	0
BB	Bb	0	0	0	1
BB	bb	10	0	0	10
Bb	BB	3	0	2	1
Bb	Bb	1	0	1	0
Bb	bb	4	5	0	4
bb	BB	0	0	0	3
bb	Bb	2	1	0	2
bb	bb	0	7	0	9
Total		21	13	6	30
Expected		20.75	13.25	5.25	30.75

The expectations here presented are based on the most probable result of each of the matings, considered on an individual basis with reference to the number of animals produced by each type of mating, but without figuring the proportions of the sexes as equal. From these data it would appear that the black-and-white color of Ayrshire cattle behaves in an ordinary sex-limited manner similar to the horns in sheep as discussed by Wood (7) and the rudimentary mammæ in swine as reported by the writer (6).

DISCUSSION

Arkell and Davenport (1) have reported on the inheritance of horns in sheep and have attempted to bring it under the ordinary sex-linked scheme of inheritance by an ingenious system of inhibitors and horn factors. Such an explanation was doubtless justified when horns in sheep were the only character known in which the reversal of dominance in the two sexes existed, but now that at least two other characters are known in which an exactly similar system of inheritance occurs, it seems unnecessary to assume the complexities hypothesized by these investigators. Instead, the much simpler and probably more perfectly descriptive explanation adopted by Wood (7) in his original paper seems more logical.

COLOR RECORD OF PROGENY IN AYRSHIRE CATTLE

The following record presents the data considered in this paper. The term "red" refers to red-and-white and the term "black" refers to black-and-white. The hereditary constitution assigned the breeding animals retained in the herd or found in the pedigrees of animals in the herd is also given.

Johanna Croft King, Bb (described as dark).
- Sir Croft of Spring City, Bb (black).
- Johanna of Juneau, bb (red).

College Marquis, Bb (described as dark).
- Marquis of Woodruff, bb (red).
- Maggie of Woodruff, Bb (red).

Woolford's Good Gift, Bb (described as mahogany).
- Lessnessock Oyama's Good Gift, Bb (described as dark).
- Pearl 3d of Woolford, bb (red cow).

Melrose Good Gift, BB (black-and-white).
- Woolford's Good, Gift Bb.
- Florence Melrose, Bb (red cow).

College Maud, bb (red).....
- Marquis of Woodruff, bb (red).
- Star of Hillview, Bb (red).

Bangora, BB (black).....
- White Prince, Bb (described as mahogany in pigmented areas).
- Star of Hillview, Bb (red).

College Marquis 2d, bb (red)
College Marquis 3d, bb (red).....
(See progeny of College Maud.)
- College Marquis, Bb (dark).
- College Maud, bb (red).

Progeny of College Maud 31350 (red), bb:
- One red heifer by unknown red bull, bb.
- One red heifer by College Marquis, Bb.
- Three red bulls by College Marquis, Bb.
- One red heifer by Johanna Croft King, Bb.
- One red heifer by Sir Croft of Spring City, Bb.
- One red heifer by Melrose Good Gift, BB.

Progeny of College Maud 2d (red), bb (daughter of College Maud by College Marquis):
- One red heifer by College Marquis, Bb.

Progeny of College Maud 2d's heifer (red), bb (daughter of College Maud 2d by College Marquis):
- One black bull by Sir Croft of Spring City, Bb.
- One red bull by College Marquis 3d, bb.
- One black bull by Melrose Good Gift, BB.
- One red heifer by Melrose Good Gift, BB.

Progeny of Kansas Croft Maud (red), Bb (daughter of College Maud by Sir Croft of Spring City):
- One red heifer by Melrose Good Gift, BB.
- One black bull by Cavalier's College Master, bb.

Progeny of Johanna Croft Maud (red), bb (daughter of College Maud by Johanna Croft King):
- One red heifer by Melrose Good Gift, BB.

Progeny of Georgie Em 25749 (red), bb:
One red heifer by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One black bull by Melrose Good Gift, BB.
One red heifer by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

Progeny of Georgie Croft (red), bb (daughter of Georgie Em by Sir Croft of Spring City):
Three black bulls by Melrose Good Gift, BB.

Progeny of Marquis Em (red), bb (daughter of Georgie Em by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.

Progeny of Johanna of Juneau 26290 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
Twins (one black bull and one red heifer) by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

Progeny of Elizabeth of Juneau 26292 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
Two black bulls by Melrose Good Gift, BB.

Progeny of Rose of Oakdale 26291 (red), bb:
Two red bulls by College Marquis 2d, bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Rosa Lee Melrose (red), bb (daughter of Rose of Oakdale by Melrose Good Gift, BB):
One red bull by Cavalier's College Master, bb.

Progeny of Canary Belle 25748 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Melrose Canary Belle, (red), Bb (daughter of Canary Belle by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

Progeny of Fearnot of Oakdale 26289 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red bull by College Marquis 2d, bb.

Progeny of Lady Marquis Fearnot (red), bb (daughter of Fearnot of Oakdale by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.

Progeny of Bangora 29700 (black), BB:
One red heifer by Marquis of Woodruff, bb.
One red heifer by College Marquis, Bb.
Two black heifers by College Marquis, Bb.
One black bull by Sir Croft of Spring City, Bb.
One black bull by Johanna Croft King, Bb.
One black heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Bangora 2d (black), BB (daughter of Bangora by College Marquis):
One black bull by Johanna Croft King, Bb.
Two black heifers by Melrose Good Gift, BB.

Progeny of Bangora's Melrose (black), BB (daughter of Bangora by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

CONCLUSIONS

(1) Black-and-white color is a simple allelomorph of red-and-white color in Ayrshire cattle.

(2) In the male the black-and-white character is dominant and in the female the red-and-white character is dominant.

(3) Males heterozygous for the two characters are black-and-white, while females heterozygous for the two characters are red-and-white.

LITERATURE CITED

(1) ARKELL, T. R., and DAVENPORT, C. B.
1912. Horns in sheep as a typical sex-limited character. *In* Science, n. s. v. 35, no. 897, p. 375–377.

(2) GEROULD, J. H.
1911. The inheritance of polymorphism and sex in Colias philodice. *In* Amer. Nat., v. 45, no. 533, p. 257–283, 5 fig. References to literature, p. 283.

(3) JACOBSON, Edward.
1909. Beobachtungen über den Polymorphismus von Papilio memnon L. *In* Tijdschr. Ent., deel 52, afl. 3/4, p. 125–157, 2 fig., tab.

(4) KUHLMAN, A. H.
1915. Black and white Ayrshires. *In* Jour. Heredity, v. 6, no. 7, p. 314–322, illus.

(5) MEIJERE, J. C. H. de.
1910. Über Jacobsons Züchtungsversuche bezüglich des Polymorphismus von Papilio Memnon L. ♀ und über die Vererbung sekundärer Geschlechtsmerkmale. *In* Ztschr. Indukt. Abst. u. Vererbungslehre, Bd. 3, Heft 3, p. 161–181, pl. 3.

(6) WENTWORTH, E. N.
1912. Another sex-limited character. *In* Science, n. s. v. 35, no. 913, p. 986.

(7) WOOD, T. B.
1905. Note on the inheritance of horns and face colour in sheep. *In* Jour. Agr. Sci., v. 1, pt. 3, p. 364–365, pl. 4.

WATERMELON STEM-END ROT

[PRELIMINARY PAPER]

By F. C. MEIER,
Student Assistant, Cotton and Truck Disease Investigations, Bureau of Plant Industry

During the last few years in certain parts of the United States shippers have been seriously troubled by a decay which attacks watermelons (*Citrullus vulgaris*) in transit and may sometimes destroy or render unsalable a large percentage of a shipment before it reaches its destination. Owing to this fact, in the season of 1915 the Department of Agriculture began a careful investigation of shipping conditions, in the course of which the present writer had an opportunity to make a laboratory study of some decayed material.

This material was taken from a shipment received in Washington, D. C., on July 24, 1915. The shipment consisted of five carloads of approximately 900 watermelons each, no one car of which yielded more than 300 salable melons, owing to the prevalence among them of the disease. The decayed watermelons were distributed through the car entirely without reference to position, a fact which made it seem manifestly impossible that the trouble could have originated from mechanical or chemical injury received from contact with the walls or the floor of the car.

This examination indicated, moreover, that, as has been reported in the case of other shipments, the injury of these watermelons had occurred in a very uniform manner. In its early stages the presence of the decay was indicated by a watery discoloration of the rind in an area closely surrounding and apparently extending from the stem. Beginning in this way there were all stages of decay up to those where about half or three-quarters of the melon were involved. In such cases the rind of this portion had become soft and wrinkled, so that in cross section it appeared much like that of the watermelons shown in the lower row of Plate XVII, figure 1. The meat below this part of the rind was slimy and blackened, while that at the opposite end of the melon remained sound, not having as yet become included in the decay. Owing to the warm, moist conditions at this season, the portion involved was covered by a gray or somewhat black mold, so that the origin of the trouble could not be readily ascertained.

An abundance of material being available at this time, an attempt was made to find out whether the injury was due to the action of some fungus, and, if this proved to be the case, to obtain the specific organism

Journal of Agricultural Research, Vol. VI, No. 4
Dept. of Agriculture, Washington, D. C. Apr. 24, 1916
dg G—76

in pure culture. In endeavoring to obtain such cultures, the following procedure was adopted. Several watermelons were selected in which the decay was just beginning to be apparent. A razor was flamed; and with this, a funnel-shaped section, which included a portion of both diseased and healthy tissue, the two being separated by a more or less distinct line of demarcation, was cut from the melon. After the razor had been flamed again, the section was divided along the line of demarcation which distinguished the advancing edge of the decay, the plug being cut from the inside toward the outer surface. This gave access to a portion of the rind to which the fungus filaments were probably just advancing and which would be unlikely to contain concomitant forms. From this region, using a sterile platinum needle, small portions were removed from just below the surface and placed directly on synthetic agar in sterile Petri dishes. After two days, during which the plates were kept at a temperature of 27° C., an abundant mycelial growth of a gray color appeared in every instance. A number of transfers of the mycelium thus obtained were made to potato cylinders, and in all cases a fungus developed which possessed the characteristics of the genus Diplodia. In order to test the capacity of this organism for producing the decay, the pure culture was inoculated into a sound watermelon at three widely separated points, at each of which the characteristic rot was reproduced.

The direct connection between this fungus and the disease having been thus indicated, 16 healthy watermelons were obtained for more inoculations. They were bought at the wharf in Washington, D. C., and came from the Pyankatank River district in Virginia, a region free from the disease, so far as is known. It may be well to mention in this connection that the decay has usually been reported as occurring on the variety known as "Tom Watson." This is probably due to the fact that in the last few years this melon has been grown somewhat to the exclusion of other varieties. Of the melons chosen for inoculation, three were "Excel" melons; the remainder were of the "Tom Watson" variety.

These melons were placed on a table near a large window which was kept open the greater part of the day, and were protected from the direct light of the sun by a cardboard screen. For a period of nine days, during which time the melons were under observation, the average temperature was 26.5° C. Of these 16 watermelons, 8, two of which were of the "Excel" variety, were inoculated with the fungus, the cultures used in this case having been derived from the original subculture. This was accomplished by making with a sterile knife at a single point near the stem an incision, into which a bit of the growing fungus mycelium was introduced. A similar wound was made in the remaining 8 melons, including the third "Excel" variety, but no infectious matter was introduced. Within 36 hours the 8 inoculated melons began to show

signs of decay, while the 8 checks remained perfectly sound throughout the course of the experiment. There was no decay present on the inoculated melons except that which originated at the point of inoculation.

The decay is first noticeable as a somewhat circular discolored area surrounding and extending from the point of inoculation. On the watermelons observed in the laboratory this area gradually increased in size until at the end of six days about half of the melon was involved. At this time the advance of the decay seemed to become less rapid and the area which was first decayed began to show a blackening due to the formation of pycnidia by the fruiting fungus. This area spread daily, and at the close of nine days the stem end of the melon presented a withered, charred appearance. Plate XVII, figure 1, is a reproduction of a photograph of nine of these melons. The four in the upper row are checks; the five below were inoculated.

The fructification of the fungus may be briefly described as follows: Pycnidia separate or confluent, smooth or, under moist conditions, covered with loose olivaceous hyphæ, 180 to 250μ in diameter. Spores 24 to 30μ by 10 to 14μ, oval, uniseptate, dark brown. On the material taken from the watermelons inoculated in Washington no paraphyses could be detected. They are present, however, when the organism is grown upon potato cylinders, a fact which would tend to support the conclusions reached by Taubenhaus,[1] to whose work reference will be made in the following paragraph.

It has long been known that those members of the Sphaeropsideae which produce brown uniseptate spores are extremely variable. The distinctions between the genera Diplodia, Botryodiplodia, Chaetodiplodia, Lasiodiplodia, and Diplodiella have been based on slight structural variations in the pycnidia. The points of separation are the relation of the pycnidia to one another, whether scattered or cespitose; their relation to the host, whether subcutaneous, erumpent, or superficial; the presence or absence of bristles and of paraphyses. These are all characteristics which one might expect to vary somewhat with the characteristics or the condition of the host. This variation probably occurs; and for this reason there has been some uncertainty as to the proper position certain species should occupy in classification. *Botryodiplodia theobromae* Pat., which causes a dieback of *Hevea braziliensis* in Ceylon, southern India, and the Malay States, is an example; and in his account of this fungus Petch[2] remarks that—

> Among the names which are known to refer to this species are *Macrophoma vestita, Diplodia cacaoicola, Lasiodiplodia theobromae, Diplodia rapax,* and there are probably others. *Botryodiplodia theobromae* is its earliest name, as far as is known, but some prefer to call it *Lasiodiplodia theobromae.*

[1] Taubenhaus, J. J. The probable non-validity of the genera Botryodiplodia, Diplodiella, Chaetodiplodia, and Lasiodiplodia. *In* Amer. Jour. Bot., v. 2, no. 7, p. 324-331, pl. 12-14. 1915.

[2] Petch, Thomas. Physiology & Diseases of Hevea braziliensis . . . 268 p., 16 pl. London, 1911.

Taubenhaus, as a result of his inoculations upon sweet potato (*Ipomoea batatas*) with *Diplodia tubericola* E. and E., *Diplodia gossypii* Zim., *Diplodia natalensis* Pole Evans, and *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl., suggests that the characteristics of the genus Diplodia be so extended that it may include all of the five genera.

This genus, although it is not thought to include forms which are absolute parasites, is nevertheless a source of serious trouble among some of our cultivated plants. The injury is usually confined to a fruit rot or to a dieback of the younger branches or shoots as in the Citrus disease prevalent in Florida and the Isle of Pines.[1] In both cases the fungus has been described as following an injury which has been previously inflicted either by mechanical means or as the result of the action of some other fungus. In the United States the more important crops which hitherto have been known to be affected are sweet potato, Citrus fruits, corn (*Zea mays*), and cotton (*Gossypium* spp.) In our Southern States the *Diplodia* injury is of considerable consequence in connection with these products. As one enters the Tropics the number of plants which are attacked increases. Among the list of hosts found here are *Citrus* spp., *Hevea* spp., *Theobroma cacao*, and *Thea* spp. In certain cases where the growing plant is attacked, the injury produced is sufficient to cause the death of the host, as is the case with *Diplodia vasinfecta* Petch, which causes an internal rootrot of tea.

Since the cotton, sweet-potato, and watermelon fields of the South are not widely separated, it is of some interest from the economic standpoint to know whether a species found on one host will grow equally well upon another. Plate XVII, figure 2, shows a watermelon nine days after it had been inoculated with a culture of *Diplodia tubericola* E. and E. obtained from Mr. L. L. Harter, of the Bureau of Plant Industry. The decay took the same course in this melon as has been described for the other inoculated material, which is shown in Plate XVII, figure 1. The pycnidia which were produced, however, retained the paraphyses.

While the *Diplodia* injury is apparently the cause of serious loss in the watermelon industry, there are other ways in which the crop suffers. Dr. W. A. Orton, Pathologist in Charge of Cotton and Truck Disease Investigations, Bureau of Plant Industry, who has made a careful study of shipping conditions, is inclined to believe that the injury is confined to certain districts. In other sections, anthracnose, due to *Colletotrichum lagenarium*, is the source of considerable trouble. To the losses thus caused by fungi must be added a small percentage of melons which have been damaged by rough treatment and by the use of cars which have been employed for the transportation of fertilizer or chemicals to the fields.

[1] Earle, F. S., and Rogers, J. M. Citrus pests and diseases at San Pedro in 1915. *In* San Pedro Citrus Path. Lab. 1st Ann. Rpt. 1915, p. 5-41, 19 fig. [1915.]

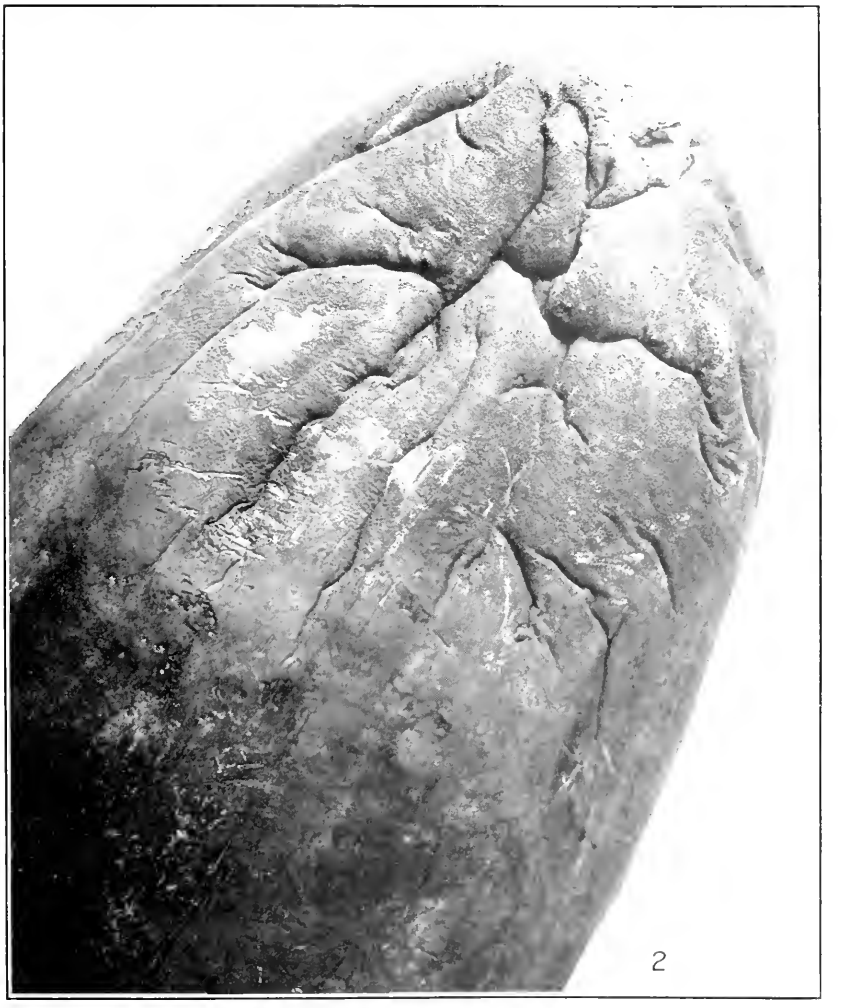
PLATE XVII

Watermelons, showing the effect of inoculation with species of Diplodia:

Fig. 1.—The upper four melons were held as checks; the lower five are melons nine days after having been inoculated with a culture of *Diplodia* sp. which had been isolated from a decaying watermelon obtained from a freight car at Washington, D. C.

Fig. 2.—A watermelon nine days after having been inoculated with a culture of *Diplodia tubericola* E. and E.

1



2

EFFECT OF PASTEURIZATION ON MOLD SPORES

By CHARLES THOM, *Mycologist, Bureau of Chemistry,* and S. HENRY AYERS, *Bacteriologist, Bureau of Animal Industry*

INTRODUCTION

Definite experiments to determine whether spores of the common saprophytic molds survive the temperatures used for the pasteurization of milk have not been reported. These spores are certainly present and are frequently abundant in ordinary market milk. Vague and general statements that such organisms do or do not survive are not uncommon, but are not supported by reference to actual work. To obtain such data studies were made with spores from pure cultures of a series of molds including several species of Penicillium, Aspergillus, and of the mucors, with, in some experiments, the addition of *Oidium* (*Oospora*) *lactis* and one strain of Fusarium. These sets of experiments were made to test, as carefully as laboratory conditions would permit, the temperatures used in pasteurization by the "holder" process, those used in the "flash" process, and the effects of dry heat.

EXPERIMENTS WITH THE HOLDER PROCESS OF PASTEURIZATION

Bacteriological studies of milk treated by the holder process have fixed the temperatures between 140° and 145° F. (60° to 62.8° C.), maintained for 30 minutes, as the minimum heating for the destruction of pathogenic organisms which may be found in milk. Although certain bacteria survive this heating it has been found that milk so treated is free from the ordinary disease-producing organisms, safe for consumption, unchanged in taste, and low enough in acid organisms to be handled with-without souring too quickly.

To study the effect of this process of pasteurization on mold spores, conidia from pure cultures of molds were first transferred to tubes of sterile water to obtain a suspension of spores. Transfers from such a suspension reduce the danger of such spores being blown by air currents into the cotton plugs and upon the walls of the test tubes used, where they might escape the full temperature applied to the milk. In the first series the inoculations were made by transferring 1 c. c. of this suspension in sterile pipettes into duplicate tubes of sterile milk. In a later series a platinum loop was used, since the tendency of the conidia to float thickly upon the surface of the water made this a quick and effective method of handling them. For most species it was thus possible to transfer spores enough to make a visible film over a part of the surface of the milk. None

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dh

Vol. VI, No. 4
Apr. 24, 1916
A—20

of the species used produced visible growth except upon or near the surface of the milk. Observations of growth must include, therefore, the surface of the milk and especially the glass from the surface of the milk upward for a few millimeters, since most molds begin to grow first upon the glass. When no spores occurred upon the glass a free-floating colony in one case escaped observation until it fruited.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. The results of the experiments with the holder process are shown in Table I. In preparing this table the records of the checks, or unheated tubes, of successive experiments were found sufficiently uniform to permit them to be averaged and appear but once. Experimental tubes were made in duplicate; and when the results were not reasonably harmonious the work was repeated. Table I summarizes the tabulated data from a series of experiments extending over a period of several months.

TABLE I.—*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6° C.) for 30 minutes* [1]

Name of mold.	Serial No.	Growth of spores when not heated (control).			Growth of spores when heated to temperature indicated and held for 30 minutes.																				
					120° F. (48.9° C.).			125° F. (51.7° C.).			130° F. (54.5° C.).			135° F. (57.2° C.).			140° F. (60.0° C.).			145° F. (62.8° C.).			150° F. (65.6° C.).		
		2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Aspergillus candidus	106	0.2	0.5	0.7	.....	.....	.....	0.1+	0.3	0.6	0.1+	0.3+	0.7	(?)	0.3+	0.6	(?)	(?)	0.4	(?)	0.0	0.0	.....	.....	.....
Aspergillus flavus (series)	108	.4	.6	.9	.....	.....	.....	.1−	.4	.9	(?)	.5	.8	(?)	.4−	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	ABC 3538	.5	.8	1.0	.....	.....	.....	.1−	.5	1.0	(?)	.5	.8	(?)	(?)	.5−	(?)	0.0	.0	(?)	.0	.0	.....	.....	.....
Do	Rg 136	.4	.9	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.1	.6	1.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	Se 171	.4	.7	.9	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.1y	.3−	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus fumigatus	118	.6	1.0	1.0	.....	.....	.....	.....	.....	.....	.1+	.9	1.0	.2	.9	1.0	.2+	.9	1.0	(?)	.3	1.0	(?)	0.2b	0.6b
Aspergillus glaucus	2705	.2	.4	.7	(?)	0.2	0.4	(?)	.2−	.3	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3512	.2	.5	.8	0.2	.4	.7	(?)	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3555.21	.2	.5	.8	(?)	.4	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus nidulans	.....	.3	.9	1.0	.....	.....	.....	.2+	.8	1.0	.1−	.4	.7	(?)	.3y	.5	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus niger (series)	111	.4	1.0	1.0	.....	.....	.....	.1+	.8	1.0	(?)	.5	1.0	(?)	.6	1.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus niger, var. *altipes*	3534a	.4	.8	1.0	.....	.....	.....	.2+	.7	1.0	.1+	.7	1.0	(?)	(?)	.4	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus niger (*cinnamomeus*)	3534b	.3	.7	1.0	.....	.....	.....	.1−	.4	.6	(?)	.3−	.8	(?)	(?)	.3	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus niger (*fuscus*)	3534c	.3	.7	1.0	.....	.....	.....	.1	.4	.8	(?)	.3+	.8	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus ochraceus	112	.3	.7	1.0	.....	.....	.....	(?)	.3	.7	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0*	(?)	.0	.0*	(,)	.0	.0
Aspergillus oryzae	113	.4	.6	.9	.....	.....	.....	(?)	.3−	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus repens	.....	.2	.5	.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.1	.5	.6	(?)	.5	.8	0.1	.5	.8	(?)	.4	.6
Aspergillus wentii	116	.2	.7	.9	.....	.....	.....	(?)	.2−	.6	(?)	.0	.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus sp	Ra 42	.3	.6	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.2	.6	1.0	(?)	.4	1.0	(?)	.0	.0	(?)	.0	.0
Do	3522.30	.4	.8	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.....	.....	(?)	.0	.0	.....	.....	.....
Do	3522.36	.2	.3	.5	.....	.....	.....	.1−	.25	.5	(?)	.2−	.3	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3556	.3	.4	.7	.....	.....	.....	.1	.2	.7	(?)	.0	.0	(?)	(?)	.2y	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Circinella sp	3514 Cl.	.3	.7	1.0	.....	.....	.....	(?)	.5	.8	(?)	.2	.7	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Mucor sp	3513	.3	.7	1.0	(*)	(*)	(*)	(?)	.2	1.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3523.6	.4	1.0	1.0	.....	.....	.....	.1−	.6	1.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3560	.3	.7	1.0	.1y	.4	1.0	.1y	.7	1.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3514 D4	.7	1.0	1.0	.....	.....	.....	.4+	1.0	1.0	.2+	1.0	1.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Rhizopus nigricans	.....	.7	1.0	1.0	(*)	(*)	(*)	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Syncephalastrum sp	.....	.6	.8	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.4	.9	1.0	.2−	.7	1.0	(?)	.0	.0	(?)	.0	.0
Fusarium sp	.....	.3	.7	.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.2	.5	.9	(?)	.5	.9	(?)	.0	.0	(?)	.0	.0
Oidium lactis	.....	.3	.5	.7	.2	.4	.7	.1	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium atramentosum	38	.2	.6	1.0	.2	.3	.8	.1−	.3	.7	(?)	.6+	.8	(?)	.4+	.7	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0
Penicillium brevicaule	2	.3	.6	1.0	.2	.4	.8	(?)	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....

[1] 1.0, a typical spore-bearing colony; 0.1, discernible germination of conidia; tenths, 0.1 to 1.0, relative amount of growth; ?, doubtful; 0, no growth; y, growth of a single spore; −, growth of a few widely scattered spores; +, growth of many spores; *, inharmonious results at times, but usually as given in the table.

TABLE I. —*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6 C.) for 30 minutes*—Continued

Name of mold.	Serial No.	Growth of spores when not heated (control).			Growth of spores when heated to temperature indicated and held for 30 minutes.																				
					120° F. (48.9° C.).			125° F. (51.7° C.).			130° F. (54.5° C.).			135° F. (57.2° C.).			140° F. (60.0° C.).			145° F. (62.8° C.).			150° F. (65.6° C.).		
		2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Penicillium camemberti	5	0.2	0.3	0.5	0.1	0.3	0.6	(?)	0.2y	0.3	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	.....	.....	.....
Penicillium camemberti, var. *rogri*	6	.2	.4	.7	.2	.4	.8	(?)	.2	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium chrysogenum	26	.3	.7	1.0	.3	.7	1.0	0.1—	.4	.8	(?)	(?)	.2—	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium citrinum	15	.2	.5	.8	(?)	.0	.0	(?)	.2—	.4	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium commune	23	.2	.4	.8	.....	.....	.....	.1	.4	.6	(?)	.2—	.3	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium cyclopium	2543a	.2	.5	.8	(?)	.2y	.4	(z)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium divaricatum	34	.3	.4	.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.15	.3+	.4	(?)	.2—	.3	(?)	.0	.0
Penicillium expansum	14	.2	.5	.7	(?)	.3	.8	(?)	.2	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium (*Citromyces*)	3523.4	.3	.5	.6	.....	.....	.....	.1	.4	.5	(?)	.3	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium granulatum	9	.2	.6	.8	.....	.....	.....	.1	.3	.6	(?)	.1y	.2	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium luteum	11	.2	.4	.7	.....	.....	.....	.1—	.3	.4	(?)	.3—	.4—	(?)	(?)	.3	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium notatum	102	.2	.5	1.0	.1	.4	.9	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium pinophilum	1	.2	.5	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum	17	.3	.5	.8	.....	.....	.....	(?)	.2	.5	(?)	.1y	.2	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium roqueforti	18	.2	.5	.8	.....	.....	.....	.1	.4	.8	(?)	.3—	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium rugulosum	46	.2	.3	.7	.....	.....	.....	.1	.3	.6	(?)	.2—	.2	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium solitum	2546	.2	.5	.7	.2	.4	.8	(?)	.3	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium spinulosum	45	.2	.4	.5	.....	.....	.....	.1—	.3	.4	(?)	.2y	.3	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium variabile	3551	.2	.5	.8	.2	.4	.9	(?)	.2—	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium viridicatum	2552	.1	.2	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	2643	.2	.4	.7	.1	.4	.8	(z)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3028	.3	.6	.9	(?)	.3	.8	(?)	.1y	.2	(?)	.1y	.3	(?)	.0	.0	(1)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3514 A2	.2	.4	.8	.2	.6	.9	.1	.5	1.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium asperulum	2683	.3	.5	.8	(?)	(?)	.3+	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	28	.2	.3	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	63	.3	.6	.9	.1	.3	.3	(?)	.2y	.5	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	103	.4	.8	1.0	.....	.....	.....	.3	1.0	1.0	0.1—	.5	1.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	2670	.2	.5	.9	.....	.....	.....	.....	.....	.....	.1	.4	.8	(?)	.2—	.6	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3525.61	.2	.5	.8	.....	.....	.....	.1+	.3	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3553	.4	.7	1.0	.....	.....	.....	.15+	.6	.9	(?)	.2—	.5	(?)	.3—	.6	(?)	.2	.8	(?)	.0	.0	.....	.....	.....
Do	3555.18	.2	.4	.7	.1+	.4	.8	.1	.5	.9	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	3555.19	.....	.....	.....	.....	.....	.....	.1	.9	1.0	(?)	.3	1.0	(?)	(?)	.3	(?)	.0	.0	.....	.....	.....	.....	.....	.....

A study of Table I shows that very few mold spores survive exposure to 140° F. (60° C.) in milk for 30 minutes and that at 145° F. (62.8° C.) still fewer are found. With reference to significant organisms, among the mucors the *Mucor racemosus* group (3513, 3523.6, 3560) and *Rhizopus nigricans*, which are found more frequently than all others of this group combined, were destroyed at 130° F. (54.5° C.). The common green species of Penicillium are mostly dead at 130° F. (54.5° C.); a few stand 135° F. (57.2° C.), but two, one of them an undescribed soil organism, survived 140° F. (60° C.) for 30 minutes. Among species of Aspergillus, however, the strains of *A. flavus*, *A. fumigatus*, and *A. repens* all survived 145° F. (62.8° C.) for 30 minutes; *A. repens* and *A. fumigatus* both survived 150° F. (65.6° C.). These three species are always found in forage and feeding stuffs; hence, milk is more or less subject to contamination with them. *A. repens* grows very poorly in milk, however, and the examination of a great many cultures of milk and its products has shown that the actual development of *A. flavus* and *A. fumigatus* is comparatively rare. Although these organisms grow at blood heat and have demonstrated their pathogenicity even to human beings at rare intervals as causes of disease in the lungs, there is no report of their growth in the alimentary canal.

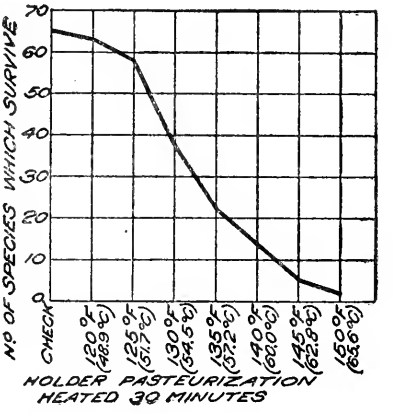


FIG. 1.—Curve of the number of species of molds surviving pasteurization of milk for 30 minutes at a series of temperatures.

The destruction of mold spores by the holder process of pasteurization is shown more clearly in figure 1, where the results have been plotted.

Pasteurization of milk at 145° F. (62.8° C.) may therefore be regarded as destroying mold spores completely enough to render them a negligible factor in the further changes found in the milk.

EXPERIMENTS WITH THE FLASH PROCESS OF PASTEURIZATION

In working with continuous pasteurizers, temperatures of 165° to 175° F. (73.9° to 79.5° C.) are reached by heating within a period of approximately 30 seconds and maintained about 30 seconds. This is followed by quick cooling. Lower temperatures have not been deemed satisfactory. A series of experiments was therefore planned to subject the freshly inoculated spores of species of Penicillium, Aspergillus, and of the mucors to these temperatures and to determine their relative ability to survive such heating. For this purpose glass tubing about 3 mm. in diameter was drawn into capillary form so that each tube had 3 or 4 inches of the original tub-

ing with 2 to 4 inches of capillary tube approximately 0.5 mm. in diameter. The open end of each tube was plugged with cotton. The tubes were packed into a copper case and dry-sterilized. For each experiment a few drops of sterile milk were transferred to the conidial surface of a colony and the conidia stirred into the milk. A column of milk 15 to 30 mm. long, bearing numerous conidia, was then drawn into the capillary tube and the end sealed in the flame. Experiments had shown that alcohol boiling at 172.4° F. (78° C.) when so treated would boil in 20 to 30 seconds when the tubes were thrust into water at 174.4° F. (79.1° C.). This showed that milk containing mold spores could be heated in from 20 to 30 seconds in capillary tubes to any given temperature when immersed in water 2 degrees Fahrenheit above the desired pasteurizing temperature. In our experiments, therefore, it was possible to duplicate flash pasteurization on a laboratory scale; for example, to pasteurize at 165° F. (73.9° C.) the capillary tubes containing milk and mold spores were held in water at 167° F. (75° C.) for 1 minute. During this period about 30 seconds were required to heat the milk and it was held at the pasteurizing temperature the other half minute. This is approximately the heating period of milk in commercial flash pasteurization. After heating for the required time, the tubes were cooled by thrusting them into cold water. The tip of the capillary was then broken off and the contents streaked upon slanted Czapek's solution agar. The slants were incubated, observed occasionally, and the results of the various experiments were tabulated separately and then brought together in Table II.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds* [1]

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 145° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
Aspergillus candidus	106	0.4	0.7	0.3	1.0	0.3	0.7	0.0	0.0	....	....	....	....	....	....
Aspergillus flavus series	108	.9	1.0	.8	1.0	.4	.8	.0	.0	0.6	1.0	0.0	0.0	0.0	0.0
Do	3538-108	.9	1.0	.6	1.0	.4	.8	.0	.0	....	....	....	....	....	....
Do	R2136	.8	1.0	.6	1.0	.5	.8	.0	.0	....	....	....	....	....	....
Do	Sc171	.7	.9	.0	.0	.5	.9	.0	.0	....	....	....	....	....	....
Aspergillus fumigatus	118	.9	1.0	.9	1.0	.3	.8	.0	.0	.6	1.0	.0	.0	.0	.0
Aspergillus globosus?	2705	.8	1.0	.0	.0	....	....	.0	.0	.4	.8	.5?	1.0?	.0	.0
Do	3512	....	....	....	....	.3	.7	.0	.0	....	....	....	....	....	....
Do	3555-21	.8	.9	.0	.0	.3	.6	.0	.0	....	....	....	....	....	....
Aspergillus nidulans	110	.3	1.0	.3	1.0	.3	.8	.0	.0	.5	.9	.0	.0	.0	.0
Aspergillus niger	111	.9	1.0	.0	.0	.3	.7	.0	.0	.6	1.0	.0	.0	.0	.0
Aspergillus niger, var. *altipes*	3534-a	.9	1.0	.6	1.0	.5	.8	?	.4?	....	....	....	....	....	....
Aspergillus cinnamomeus	3534-b	.8	1.0	.6	1.0	.5	1.0	.0	.0	.5	1.0	.0	.0	.0	.0
Aspergillus fuscus	3534-c	.8	1.0	.7	.9	....	....	.0	.0	.7	1.0	.0	.0	.0	.0
Aspergillus ochraceus	112	.9	1.0	.0	.0	.9	1.0	.0	.0	....	....	....	....	....	....
Aspergillus oryzae	113	.8	1.0	.0	.0	.9	1.0	.0	.0	.5	.8	.0	.0	.0	.0

[1] 1.0, a typical colony; decimals, proportionate growth; 0.0, no growth; ?, inharmonious results.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds*—Continued

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 145° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
Aspergillus repens	.....	.....	.....	.....	.....	0.8	1.0	0.8	1.0	.....	.....	.....	.....	.....	.....
Aspergillus wenti	116	.8	1.0	.0	.0	.5	1.0	?	.5?	.....	.....	.....	.....	.....	.....
Aspergillus sp	Ra42	.7	1.0	.6	1.0	.8	1.0	.0	.0	.5	.8	.0	.0	.0	.0
Do	3522.30	.9	1.0	.0	.0	.9	1.0	.0	.0	.....	.....	.....	.....	.....	.....
Do	3522.36	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	3556	.9	1.0	.0	.0	.5	1.0	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus parasiticus	3559	.9	1.0	.6	1.0	.5	1.0	?	.6?	.7	1.0	.0	.0	.0	.0
Do	3565	.9	1.0	.5	1.0	.7	.9	.0	.0	.....	.....	.....	.....	.....	.....
Circinella umbellata	3514.C1	.8	1.0	.8	1.0	.5	1.0	.0	.0	.8	1.0	.0	.0	.0	.0
Mucor racemosus (group)	3513	.8	.8	.8	1.0	.9	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
Do	3523.6	.....	.....	.....	.....	.9	1.0	.0	.0	.....	.....	.....	.....	.....	.....
Do	3560	1.0	1.0	1.0	1.0	.....	.....	.0	.0	.9	1.0	.0	.0	.0	.0
Rhizopus nigricans	3Rn.	.8	1.0	.0	.0	.6	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
Syncephalastrum sp	Syn.	.9	1.0	.8	1.0	.....	.....	.0	.0	.....	.....	.....	.....	.....	.....
Fusarium sp	.....	.8	1.0	.9	1.0	.5	1.0	?	.7	.....	.....	.....	.....	.....	.....
Penicillium atramentosum	38	.8	1.0	9.0	1.0	.....	.....	.....	.....	.5	1.0	.0	.0	.0	.0
Penicillium biforme	39	.9	1.0	.0	.0	.3	.6	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium brevicaule	2	.3	bact.	.0	.0	.3	.6	.0	.0	.5	.6	.0	.0	.0	.0
Penicillium camemberti	5	.9	1.0	.0	.0	.9	1.0	.0	.0	.3	.7	.0	.0	.0	.0
Penicillium camemberti, var. *rogeri*	6	.8	1.0	.0	.0	.4	.6	.0	.0	.4	.9	.0	.0	.0	.0
Penicillium chrysogenum	26	.8	1.0	.0	.0	.4	.6	.0	.0	.0	.0	.0	.0	.0	.0
Penicillium citrinum	15	.7	1.0	.6	.7	.....	.....	.0	.0	.9	1.0	.0	.0	.0	.0
Penicillium commune	23	.8	1.0	.6	.8	.9	1.0	.0	.0	.7	1.0	.6?	1.0	.0	.0
Penicillium cyclopium	2543-a	.8	1.0	.4	.8	.5	.6	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium digitatum	16	.6	1.0	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium divaricatum	34	.9	1.0	.9	1.0	.4	.6	.0	.0	.8	.9	.0	.0	.0	.0
Penicillium duclauxi	20	.8	1.0	.4	.5	.5	.9	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium expansum	14	.....	.....	.....	.....	.5	.9	.0	.0	.6	.8	.0	.0	.2?	.5?
Penicillium (*Citromyces*) sp	3523.4	.9	1.0	.0	1.0	.5	.9	.0	.0	.4	.8	.0	.0	.0	.0
Penicillium granulatum	9	.9	1.0	.0	.0	.5	.9	.0	.0	.4	1.0	.0	.0	.0	.0
Penicillium italicum	10	.4	.9	.5	1.0	.3	.6	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium luteum	11	.8	.9	.4	1.0	.4	.8	Very slow.		.5	.9	?	?	.0	.0
Penicillium notatum	102	.6	.7	.1	.5?	.3	.8	.0	.0	.3	.7	.0	.0	.0	.0
Penicillium oxalicum	105	.8	.9	?	.7?	.5	.8	.4	.8	.9	.0	.0	.0	.0	.0
Penicillium pinophilum	1	.4	.8	.4	.8	.4	.9	.0	.0	.3	.7	.0	.0	.0	.0
Penicillium puberulum?	2683	.8	1.0	.0	.0	.4	.9	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum	17	.9	1.0	.0	.0	.3	.7	.0	.0	.6	.9	.0	.0	.0	.0
Penicillium purpurogenum, var. *rubri sclerotium*	2670	.....	.....	.....	.....	.3	.6	.0	.0	.5	1.0	.3	.5	.0	.6?
Penicillium roqueforti	18	.....	.....	.....	.....	.4	.8	.0	.0	.5	.9	.0	.0	.0	.0
Penicillium rugulosum	46	.4	.8	.4	.8	.....	.....	.....	.....	.3	.4	.0	.0	.0	.0
Penicillium solitum	2546	.8	1.0	.0	.0	.3	.6	.0	.0	.8	1.0	.0	.0	.0	.0
Penicillium solitum?	66	.9	1.0	.6	1.0	.5	.9	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium spinulosum	45	.8	1.0	?	.5	.....	.....	.....	.....	.5	.8	.0	.0	.0	.0
Penicillium stoloniferum	27	.9	1.0	.4?	1.0?	.4	.9	.0	.0	.5	.8	.0	.0	.0	.0
Penicillium variabile	3551	.9	1.0	.0	.0	.3	1.0	?	.7	.8	1.0	.0	.0	.0	.0
Penicillium viridicatum	2552	.9	1.0	?	.6	.3	.9	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium viridicatum, var.?	2643	.9	1.0	.7	.8	.3	.7	.0	.0	.5	.7	.0	.0	.0	?
Do	3028	.7	1.0	.0	.0	.3	.8	.0	.0	.7	1.0	.8?	.9?	.0	.0
Do	3514-a	.8	1.0	.4?	.6?	.4	.6	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium (*Citromyces*) sp	28	.....	.....	.6	.8	.3	.6	.0	.0	.4	.9	.0	.0	.0	.0
Do	63	.9	1.0	.0	.0	.4	.7	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium sp	3525.61	.8	1.0	?	.4	.4	.8	.0	.0	.....	.....	.....	.....	.....	.....
Do	3553	.....	.....	.....	.....	.5	.9	.0	.0	.6	.9	.0	.0	.0	.0
Do	3555.18	.....	.....	.....	.....	.....	.....	.....	.....	.4	.7	.0	.0	.0	.0
Do	3555.19	.7	.9	.4	.7	.6	.9	.0	.0	.....	.....	.....	.....	.....	.....

From Table II it is seen that very few of the forms are killed in 30 seconds at 145° F. (62.8° C.); nearly all, however, are destroyed at 155° F. (68.3° C.). None of the colonies found at 165° F. (73.9° C.) and 175° F. (79.5° C.) were produced in both tubes. The chance of error is not fully eliminated in these cases. The consistent character of the whole table and the innocuous character of the few organisms in which occasional colonies occurred after heating show that temperatures of 165° to 175° F. (73.9° to 79.5° C.) for 30 seconds do practically destroy the spores of these molds as they may be found in milk, although a few conidia in some species may occasionally survive.

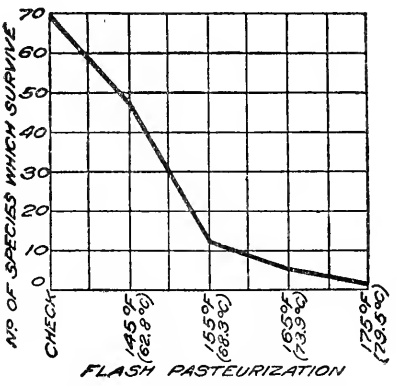


FIG. 2.—Curve of the number of species of molds surviving flash pasteurization at a series of temperatures.

Figure 2 shows graphically the effect of the flash process of pasteurization on mold spores.

DESTRUCTION OF MOLD SPORES BY DRY HEAT

The third series of experiments was planned to find the relative ability of the spores of approximately the same organisms to endure heating in dry air for the same period as used for heating in milk. After some experimentation the following method was used: Strips of heavy filter paper were cut wide enough so that only the edges would come into contact with the glass when dropped into test tubes. A drop of sterile water carrying a suspension of the spores under experiment was deposited in the middle of the paper strip and allowed to evaporate overnight. The tubes were then immersed in liquid heated to the desired temperature and held 30 minutes after check tubes carrying thermometers indicated that the air in the tubes had reached the same degree. The tubes were then removed and cooled. Melted agar was allowed to run into each tube to form a slant and the cultures were set away at room temperature. Observations of growth were made as in the previous experiments and the results tabulated in the same manner in Table III.

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*[1]

Name of mold.	Serial No.	Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes.																					
		Not heated (control) 5 days.	Heated to 180° F. (82.2° C.) 5 days.	Not heated (control) 7 days.	Heated to 190° F. (87.8° C.).		Not heated (control).		Heated to 200° F. (93.3° C.).		Not heated (control).		Heated to 210° F. (98.9° C.).		Heated to 220° F. (104.5° C.).		Not heated (control).		Heated to 230° F. (110.0° C.).		Not heated (control).		Heated to 250° F. (121.1° C.).	
					3 days.	7 days.	4 days.	8 days.	4 days.	8 days.	4 days.	7 days.	4 days.	7 days.	4 days.	7 days.	3 days.	6 days.	3 days.	6 days.	4 days.	8 days.	4 days.	8 days.
Aspergillus candidus	106	0.5	0.3	0.4	.....	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	?	0.6	?	?0.0	?	0.4	0.0	0.0
Aspergillus flavus, var.	108	.7	.7	.....	.....	.....	0.3	.....	.....	0.1	0.7	.....	0.2	0.9	.....	.....	0.2	.9	0.3	.....	0.5	.8	.0	.0
Do	3538.108	.5	.5	.....	.....	.....	.....	.....	.....	.....	.5	.....	?	.5	.....	.....	.3	.9	.5	1.0	.5	.8	?	.6?
Do	Rg136	.8	.6	.8	.....	.3	.4	.....	.....	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	Sc171	.5	.4	.....	.....	.....	.4	.....	0.3	.4	.4	.....	.....	.....	.....	.....	.2	.9	.0	.0	.....	.....	.....	.....
Aspergillus fumigatus	118	.8	.6	.....	.....	.....	.3	.....	?	.6	.5	.....	.3	.8	0.3	.....	.2	.9	.3	1.0	.5	.9	.0	.0
Aspergillus globosus	2705	.6	.4	.....	.....	.....	.2	.....	?	.2	.8	.....	.0	.0	.....	.....	.2	.8	.0	.0	.3	.7	.0	.0
Aspergillus globosus ?	3512	.8	.4	.8	.....	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	?	.9	.2	1.0	.4	.9	.0	.0
Aspergillus versicolor ?	3555.21	.5	.5	.8	.....	.0	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.2	.9	?	?	.3	.6	.0	.0
Aspergillus nidulans	110	.5	.5	.....	.....	.....	.2	.....	?	.4	.6	.....	.0	.0	.....	.....	.3	.9	?	?	.4	.8	.0	.0
Aspergillus niger	111	.7	.5	.....	.....	.....	.4	.....	?	.3	.8	.....	.0	.0	.....	.....	.3	.9	.5	.8	.....	.....	.....	.....
Aspergillus niger, var. *altipes*	3534a	.8	.6	.....	.....	.....	.3	.....	?	.5	.....	.....	.....	.....	.....	.....	.2	1.0	.6	1.0	.4	.8	.0	.0
Aspergillus cinnamomeus	3534b	.7	.3	.....	.....	.....	.3	.....	?	.4	.8	.....	?	.....	.3	.....	.2	.9	.6	.8	.4	.7	.0	.0
Aspergillus fuscus	3534c	.8	.....	.....	.....	.....	.5	.....	?	.4	.7	.....	.0	.0	.....	.....	.2	.9	.5	1.0	.4	.7	.0	.0
Aspergillus ochraceus	112	.7	.4	.8	.....	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.3	.9	?	?	.....	.....	.....	.....
Aspergillus oryzae	113	.8	.5	.....	.....	.....	.3	.....	?	.4	.8	.....	.0	.0	.2	.....	.3	.7	.0	.0	.....	.....	.....	.....
Aspergillus repens	.....	.4	.2	.....	.....	.....	.....	.....	.....	.....	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.2	.4	.0	.0
Aspergillus wentii	116	.7	.0	.7	.....	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Aspergillus sp	Ra42	.6	.3	.5	.....	.3	.2	.....	.0	.0	.....	.....	.....	.....	.....	.....	.2	.8	.0	.0	.....	.....	.....	.....
Do	3522.30	.8	.4	.....	.....	.....	.2	.....	.4	.5	.7	.....	.0	.0	.....	.....	.2	.8	?	.3	.3	.6	.0	.0
Do	3522.36	.5	.2	.5	.....	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	3556	.9	.6	.....	.....	.....	.4	.....	?	.6	.8	.....	.0	.0	.....	.....	.3	.9	.3	.5	.4	.8	.0	.0
Aspergillus parasiticus	3509	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.3	.9	.3	1.0	.4	.7	.0	.0
Aspergillus sp	3565	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.3	.9	.3	1.0	.4	.7	.0	.0
Circinella umbellata	3514 C1	.....	.....	.....	.....	.....	.3	.....	?	.4	.6	.....	?	.6	.3	0.7	.5	.9	.5	.8	.3	.7	?	.4
Mucor racemosus ?	3513	.....	.....	.....	.....	.....	.....	.....	.....	.....	.4	.....	.4	.8	.....	.5	.4	.9	.7	1.0	.4	.7	?	.8
Do	3523.6	.6	.5	.....	.....	.....	.3	.....	.3	1.0	.6	.....	?	.7	.....	.....	.4	.9	.9	.8	.4	.8	.0	.0
Do	3560	.7	.....	.....	.....	.....	.3	.....	.4	.4	.8	0.9	?	.9	.8	.8	.5	1.0	.9	.9	.6	1.0	?	1.0
Rhizopus nigricans	.....	.6	.6	.....	.....	.....	.3	0.5	.3	.5	.3	.9	.3	.9	.3	.7	.3	.8	.6	.9	.3	.6	.0	.0
Syncephalastrum sp	.....	.5	.5	.....	.....	.....	.2	.....	?	.4	?	.9	?	.6	?	?	.2	.9	?	.5	.....	.....	.....	.....
Fusarium sp	.....	.7	.5	.....	.....	.....	.5	.8	.4	.8	.....	.7	.5	.8	.....	.....	.2	.8	?	.6	.4	.6	.0	.0

[1] 1.0, a typical colony: decimals, proportionate growth, 0.0, no growth; ? inharmonious results: y, growth of a single spore,

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*—Contd.

Name of mold.	Serial No.	Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes.																					
		Not heated (control) 5 days.	Heated to 180° F. (82.2° C.) 5 days.	Not heated (control) 7 days.	Heated to 190° F. (87.8° C.),		Not heated (control).		Heated to 200° F. (93.3° C.).		Not heated (control).		Heated to 210° F. (98.9° C.).		Heated to 220° F. (104.5° C.).		Not heated (control).		Heated to 230° F. (110.0° C.).		Not heated (control).		Heated to 250° F. (121.1° C.).	
					3 days.	7 days.	4 days.	8 days.	4 days.	8 days.	4 days.	7 days.	4 days.	7 days.	4 days.	7 days.	3 days.	6 days.	3 days.	6 days.	4 days.	8 days.	4 days.	8 days.
Penicillium atramentosum	38	0.7	0.5	0.9	0.3	0.6	0.4	.....	0.0	0.0	.....	.....	.....	.....	.....	.....	?	0.8	0.0	0.0	.....	.....	.....	.....
Penicillium biforme	37	.....	.....	.....	.....	.....	.6	.....	.0	.0	0.7	.....	0.0	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium brefeldii	2	.8	.0	.7	.0	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium camemberti	5	.7	.3	.8	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium camemberti var. *rogeri*	6	.6	.3	.8	?	.2	.7	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium chrysogenum	26	.9	.8	.....	.....	.....	.7	.....	?	.7?	.8	.....	.4	.6	.....	.....	.3	.9	.4	1.0	.5	.9	.0	.0
Penicillium citrinum	15	.8	.0	.9	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium commune	23	.8	.4	.7	.0	.0	.6	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium cyclopium	2543a	.8	.....	.....	.....	.....	?	.....	?	.2	.8	.....	.0	.0	.....	.....	.2	1.0	?	?	.4	.9	.0	.0
Penicillium digitatum	16	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium divaricatum	34	.7	.6	.....	.....	.....	.3	.....	?	.6	.8	.....	?	.3	.....	.....	.2	1.0	.7	1.0	.3	.7	.0	.0
Penicillium duclauxi	20	.6	.5	.....	.....	.....	.3	.....	?	.2	.5	.....	.4	.7	.....	.....	.3	.9	?	?	.....	.....	.....	.....
Penicillium expansum	14	.....	.....	.7	?	.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium glaucum (*Citromyces*)	3523.4y	.9	.6	.9	?	.2	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium granulatum	9	.7	.....	.9	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium italicum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.3	.9	.0	.0	.....	.....	.....	.....
Penicillium luteum	11	.5	.4	.3	?	.3	.3	.....	?	.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium notatum	102	.8	.6	.7	?	.4	.6	.....	?	1.0	.8	.....	.4	.8	.0	.0	.2	.9	?	?	.....	.....	.....	.....
Penicillium oxalicum	103	.8	.7	.....	.....	.....	.7	.....	?	.4	.8	.....	.4?	.8	.....	.....	.....	.....	.....	.....	.4	.8	.0	.0
Penicillium pinophilum	1	.5	.4	.....	.....	.....	.2	.....	?	.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium puberulum	2683	.....	.....	.8	?	.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum	17	.7	.3	.7	.1	.4	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum, var. *rubri-sclerotium*	2670	.....	.....	.6	.4	.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium roqueforti	18	.7	.4	.6	.0	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium rugulosum	46	.5	.3	.8	?	.4	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium solitum	2546	.7	.4	.8	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium solitum ?	66	.....	.....	.....	.....	.....	.6	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium spinulosum	45	.5	.3	.....	.....	.....	.5	.....	?	.3?	.7	.....	.1	.5	.....	.....	.3	.9	.6	1.0	.8	.8	.0	.0
Penicillium stoloniferum	27	.....	.....	.8	.0	.0	.7	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium variabile	3551	.7	.5	.....	.....	.....	.5	.....	?	.3	.7	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

Penicillium viridicatum	2552	.9	.4	....	....	.5	....	?	.4?	.8	....	?	.6	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Penicillium viridicatum, var. ?	2643	.8	.3	.8	?	.3	.6	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3028	.8	.3	.7	.0	.0	.5	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3511 A_2	.9	.5	.9	?	.3	.6	?	.0	.8	....	.3	.8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	40002	....	....	....	.5	.4	.5	?	.4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Penicillium (*Citromyces*) sp.	28	.5	.2	.8	.0	.4	.4	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	63	.9	.5	.9	.0	.4	.7	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Penicillium sp.	3525.61	....	....	.8	?	.4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3553	1.0	.5	.8	?	.3	.6	?	.2	.6	....	.4	.5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3555.18	.8	.5	.8	?	.3	.5	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3555.19	....	....	....	....	....	.8	?	.3	.8	....	.4	.6	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

A study of Table III shows that mold spores possess much greater ability to withstand dry heat than heating in milk. Very few forms were destroyed at 180° F. (82.2° C.), but they include *Penicillium brevicaule*, which has a thick-walled spore and in laboratory cultures has remained viable at least 7 years. Only a few species of Penicillium survived heating to 200° F. (93.3° C.) for 30 minutes. All these are forms which grew at 98.6° F. (37° C.), and some of them are widely distributed.

Aside from *A. wentii*, all the species of Aspergillus survived heating at 200° F. (93.3° C.). Several of them survived at 230° F. (110° C.), but after 250° F. (121.1° C.) for 30 minutes no species showed growth after 6 days' incubation. Three of six mucors, however, survived the heating to 250° F. (121.1° C.) for 30 minutes. These species were killed quickly by both forms of heating in milk. The results of these experiments are plotted in figure 3.

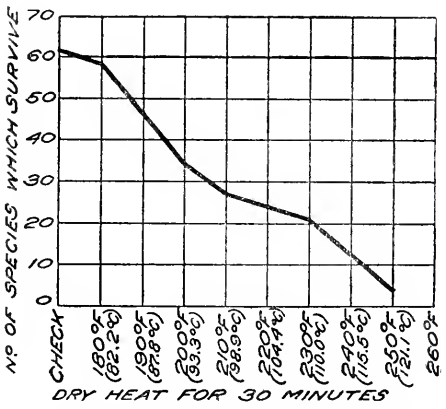


FIG. 3.—Curve of the number of species of molds surviving dry heat for 30 minutes at a series of temperatures.

The destruction of mold spores by dry heat has no relation to the subject of pasteurization of milk, but it is of scientific interest.

DISCUSSION OF RESULTS

These results with mold spores agree in general with bacteriological studies of pasteurization. Very few of these organisms found in milk survive after 30 minutes' heating to 145° F. (62.8° C.). Certain molds, notably *Aspergillus fumigatus* and *A. flavus*, do survive, but they occur only occasionally in milk. *Oidium lactis* and the mucors, which are probably more important as milk-borne organisms than all the rest, are destroyed at the low temperatures used in the holder process of pasteurization. In the flash process very few mold spores survived at 165° F. (73.9° C.). Occasionally some spores seem to have escaped destruction at 175° F. (79.5° C.), but the organisms surviving in these cases were of minor importance in the decomposition of dairy products. In confirmation of these results the writers have had access to unpublished data of Mr. R. O. Webster, of the Bureau of Chemistry, giving cultural analysis of butter made from flash-pasteurized cream on a commercial basis. Cultures from this butter showed no mold spores, while cultures made at the same time from country butter showed 20,000 to 60,000 per gram.

Mold spores in milk seem, therefore, to be destroyed completely or reduced to negligible numbers by both of the standard pasteurization processes.

Careful study of the cultures showed that the first effect of heating was to delay germination. This is indicated in the tables by the reports of successive examinations of the same culture. In Table I three reports are given; later only two reports. The third and fourth observations, however, were usually made. At times heating to a degree just under the death point delayed germination almost the full length of the usual growing period of the species. The number of possible sources of error was so great that the results of observations have been tabulated and compared. When essential harmony of results was not obtained, the work was repeated. In a few cases the continued lack of consistent results for particular organisms is indicated by the interrogation point in the tables. Even with these precautions the data obtained can be said to apply only to the strains used. This is indicated by comparing the results given for the *Aspergillus flavus* group or for the four members of the *A. niger* group. These results do not prove that other strains of these groups would respond exactly as here tabulated. In fact, more extended studies (as yet unpublished) of these two groups indicate that organisms otherwise undistinguishable may differ greatly if we measure a single physiological reaction. Such quantitative differences may persist in continued cultures, but are hardly comparable to differences in the kind of reaction as a basis for separating species. Inside the race or strain, conidia transferred from the same culture respond very differently. There is frequently a survival of a few spores where a majority of the spores die. There may be, therefore, a difference of as much as 20° F. (11.1 C.) between the temperature at which an occasional culture is completely killed and that at which cultures of that species are uniformly killed. These results resemble those obtained in determining the thermal death point of bacteria.

The applicability of these results to the occurrence of mold spores in substances other than milk has not been tested. The variation in composition of the substratum together with the heating may at times introduce a considerable variation. In general, however, it is clear that mold spores are easily killed by heat when suspended in fluid. The tables have been studied in an attempt to correlate resistance with size of spore or thickness of spore wall. No such correlation has been found. There is, therefore, no suggestion as to the nature of the difference in these organisms which affects their resistance to heat.

SUMMARY

(1) The holder process of pasteurization, in which milk was heated to 145° F. (62.8° C.) and maintained at that temperature for 30 minutes, killed the conidia of every species investigated, except those of *Asper-*

gillus repens, *A. flavus*, and *A. fumigatus*. The molds which survive are found only occasionally in milk.

(2) The flash process of pasteurization, where milk was heated to 165° F. (73.9° C.) for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175° F. (79.5° C.) only occasional spores of two forms developed.

(3) When the heating process was performed in dry air for a period of 30 seconds at 200° F. (93.3° C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of the mucors. A temperature of 250° F. (121.1° C.) over a period of 30 minutes killed all the forms of *Penicillium* spp. tried, but left an occasional living spore in one species of Aspergillus and three out of six mucors.

EFFECT OF WATER IN THE RATION ON THE COMPOSITION OF MILK

By W. F. TURNER, R. H. SHAW, R. P. NORTON, and P. A. WRIGHT, *of the Dairy Division, Bureau of Animal Industry*

INTRODUCTION

Experiments conducted at Brownsville, Tex., by the Dairy Division of the Bureau of Animal Industry indicate that the feeding of prickly-pear (*Opuntia* spp.) lowers the percentage of fat in milk. In comparison with other feeds prickly-pear contains a large amount of water and mineral matter. It was thought by the writers that one or both of these constituents might be responsible for the reduction in fat percentage; consequently experiments were conducted at Beltsville, Md., to determine the influence of the water. Work with the mineral matter is now in progress.

The literature dealing with the effects of watery feeds or water in the ration upon the quantity and the quality of milk produced contains many conflicting statements. No doubt the difficulty of eliminating all factors except the watery character of the ration is largely responsible for the conflicting nature of these statements.

Gilchrist (1)[1] reports very little difference, if any, in quantity and quality between the milk produced by cows either on pasture only or on a daily ration of mangels in varying amounts up to 86 pounds per cow and that produced by the same cows on a ration of hay and grain.

Tangl and Zaitschek (12) state, as the result of extensive experiments to determine the influence of watery feeds on milk secretion, that there is no difference between the composition of the milk from cows fed on a watery ration and that from cows fed on a dry one. They state that it is not true that watery feeds cause the production of thinner milk than dry feeds.

Lauder and Fagan (10, p. 9) reached the following conclusions from experiments extending over a 3-year period, using 60 cows and feeding a large ration of turnips (*Brassica rapa*) to compare with a dry or concentrated ration:

> The feeding of a ration containing a large quantity of water does not increase the percentage of water in the milk or reduce the percentage of fat.
>
> The greater yield of milk was obtained from the cows on the concentrated ration. On the other hand, the milk from the cows on the turnip ration contained a higher percentage of fat, and a greater total weight of fat was secreted in the milk.

[1] Reference is made by number to "Literature cited," p. 177–178.

Journal of Agricultural Research, Dept. of Agriculture, Washington, D. C.
di

Vol. VI, No. 4
Apr. 24, 1916
A—21

Holtsmark (6) reports that there is no decrease in the fat content of the milk of cows on a liberal daily ration of concentrated feed and cut straw, with as much as 77 pounds of turnips per head, after this ration is substituted for one consisting of hay, straw, concentrates, and a small quantity of roots.

A writer in the Journal of the Board of Agriculture (3), London, England, concludes from a study of the work of various investigators that, although many feeds have a specific effect on the yield and quality of milk, it may be attributed to stimulating substances in the feeds rather than to water content. These substances have a physiological rather than a nutritive effect and are present in feeds in small quantities only.

As the result of a number of experiments conducted and a review of previous work of the same character, Jordan (8, p. 69) states that, "Contrary to a notion held by many, it is not possible to water a cow's milk through her drink or through the ingesting of watery feed."

The Journal of the Board of Agriculture, London (2), reports that a dairyman was convicted in the French courts for selling adulterated milk. The conviction was based upon the assumption that it is possible to water milk either by feeding cows on watery feeds, by causing them to drink water in large quantities, or by making them drink immediately before milking. To prove the fallacy of this assumption, the Board conducted experiments with a number of cows. After feeding them an excess of common salt (sodium chlorid), or limiting the water drunk after free access to it, or permitting them to drink only immediately before milking, it was found that no change is produced in the composition of the milk.

At Offerton Hall, Durham, England, a series of experiments was conducted to determine how the composition of milk is affected by feeding wet brewers' grains. The first of these experiments (7, p. 35) indicates that the feeding of these grains to cows whose milk is habitually low in butter fat is not to be recommended, especially during the earlier stages of the lactation period, when the grains tend slightly to reduce the yield of fat. The writer advises dairymen to use such grains sparingly. Later experiments (13, p. 19–20) indicate that the grains may be fed safely if the ration contains other feeds also, and that there is no appreciable lowering of the butter fat when the grains are fed in moderate quantities.

In a general article upon the effect of different feeds upon the quality of milk, McConnell (11) says:

> It is a matter of common knowledge that the lush grass of spring, an excess of mangolds, or too many brewers' grains will promote a great flow of milk, but that that milk will be poor, and farmers who do not do anything to modify such feeding will find their milk coming dangerously near the "standard."

Hansson (4), of the Stockholm Agricultural Experiment Station, in a review of the work of various investigators concerning the effect of different feeds upon the fat content of milk, concludes that there are on

this point distinct differences among different feeds, but that the effect of any feed depends upon the composition of the other components of the ration. He states that roots have a favorable effect upon milk secretion, but tend slightly to lower the fat content.

Koch (9) reports extensive feeding experiments at Rosenhof in which cows were fed beet roots (*Beta vulgaris*), and gives the following conclusions:

> An increase in fat units (total fat) with beet-root feed, an increase of the amount of milk combined with a decrease in the fat content. However, the increase in quantity exceeded the decrease in quality so much that the cows gave 6 per cent more total fat on the beet-root ration.[1]

PLAN OF INVESTIGATION

The experimental work to determine the effect of water in the ration upon the composition of milk was conducted at the Dairy Division farm, Beltsville, Md., and included parts of three different lactation periods. The four following methods for supplying rations of widely different water content were tried:

1. A full allowance of drinking water as compared with a limited supply, the ration otherwise being alike in both cases.
2. A heavy ration of turnips as compared with a dry-roughage one.
3. Wet beet pulp as compared with dry beet pulp.
4. Green crimson clover (*Trifolium incarnatum*) as compared with the cured hay.

As the change in the fat content of the milk noted during the prickly-pear experiments took place within a few days after the change in the character of the ration and continued throughout the 80-day period, it was decided that for this work two 10-day periods of feeding any one ration, with a 10-day transition period intervening, and equal periods of feeding the comparative ration, would give time enough for any change in the composition of the milk to take place. In each series of experiments the milk from each cow was weighed at each milking, and 10-day composite samples were taken for analysis. The data obtained from each series of experiments are given separately.

FULL VERSUS LIMITED ALLOWANCE OF WATER

In this series of experiments eight cows were used and all received the same general treatment. For the first two 10-day periods the animals were given water ad libitum twice daily. Then a definite quantity of water, not more than 75 per cent of the full allowance, and in some cases less than 65 per cent, was given for two 10-day periods following a 10-day transition period. The quantity of water given in the limited water ration was so reduced that, when watered once a day, all cows drank the quantity allowed. After a second 10-day transition period, a full allowance of water was again given for two 10-day periods. This completed the work

[1] Authors' translation.

with all but two cows, which were given a still more reduced allowance of water following the second full-allowance period. Table I gives the results for each cow.

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*

COW 100

Water allowance.	Total milk.	Total water.	Fat.		Specifie gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Percent.*
Full	220. 6	412. 5	4. 50	9. 93	1. 033	9. 11	86. 39	0. 720	3. 35
Do	240. 6	502. 0	4. 50	10. 83	1. 032	9. 19	86. 31	. 710	3. 35
Transition	205. 3	340. 0	4. 60	9. 44	1. 033	9. 27	86. 13	. 720	3. 36
Limited	198. 8	340. 0	4. 80	9. 54	1. 034	9. 36	85. 84	. 710	3. 52
Do	199. 3	340. 0	4. 70	9. 37	1. 033	9. 18	86. 12	. 705	3. 53
Transition	197. 6	434. 0	4. 35	8. 60	1. 032	9. 11	86. 54	. 700	3. 41
Full	172. 2	378. 0	4. 90	8. 44	1. 032	9. 01	86. 09	. 745	3. 68
Do	167. 0	358. 0	4. 70	7. 85	1. 033	9. 18	86. 12	. 747	3. 66
Transition	149. 8	200. 0	4. 88	7. 19	1. 033	9. 36	85. 76	. 755	3. 80
Limited	135. 8	205. 0	4. 90	5. 65	1. 032	9. 30	85. 80	. 770	3. 72
Do	138. 0	215. 0	4. 80	6. 62	1. 032	9. 13	86. 07	. 750	3. 61
Average:									
Full	200. 1	412. 5	4. 65	9. 26	.....	9. 12	86. 23	. 730	3. 51
Limited	168. 0	275. 0	4. 80	7. 79	.....	9. 24	85. 96	. 734	3. 59

COW 21

Full	191. 8	500. 5	6. 00	11. 51	1. 036	9. 98	84. 02	. 770	3. 92
Do	188. 0	502. 0	6. 00	10. 88	1. 035	10. 14	83. 86	. 770	3. 94
Transition	179. 0	320. 0	6. 25	11. 19	1. 036	10. 18	83. 57	. 750	3. 94
Limited	175. 2	320. 0	6. 10	10. 69	1. 035	10. 11	83. 79	. 740	3. 87
Do	179. 0	320. 0	6. 03	10. 79	1. 035	10. 21	83. 76	. 740	3. 96
Transition	181. 6	496. 0	5. 50	9. 99	1. 034	9. 91	84. 59	. 730	3. 82
Full	184. 5	462. 0	5. 73	10. 57	1. 035	10. 00	84. 27	. 720	3. 90
Do	180. 4	473. 0	6. 00	10. 82	1. 035	9. 81	84. 19	. 740	3. 99
Transition	172. 9	250. 0	5. 95	10. 29	1. 035	9. 95	84. 07	. 755	3. 91
Limited	157. 8	255. 0	6. 00	9. 47	1. 034	10. 10	83. 90	. 750	3. 91
Do	151. 5	280. 0	5. 80	8. 79	1. 033	9. 82	84. 38	. 730	3. 76
Average:									
Full	186. 2	484. 0	5. 93	10. 94	.....	9. 98	84. 08	. 750	3. 94
Limited	165. 9	294. 0	5. 98	9. 94	.....	10. 06	83. 96	. 740	3. 88

COW 19

Full	220. 5	520. 0	5. 30	11. 69	1. 036	9. 83	84. 87	. 770	3. 73
Do	228. 6	492. 0	5. 20	11. 89	1. 035	9. 82	84. 98	. 745	3. 86
Transition	213. 0	300. 0	5. 18	11. 03	1. 036	10. 09	84. 73	. 735	3. 73
Limited	213. 1	345. 0	5. 42	11. 55	1. 035	9. 66	84. 92	. 750	3. 77
Do	202. 5	305. 0	5. 33	10. 79	1. 035	9. 98	84. 69	. 780	3. 82
Transition	203. 3	622. 0	5. 60	11. 38	1. 034	9. 66	84. 74	. 765	3. 83
Full	198. 5	520. 0	5. 50	10. 92	1. 034	9. 74	84. 76	. 775	3. 88
Do	193. 2	520. 0	5. 25	10. 14	1. 034	9. 71	85. 04	. 765	3. 74
Average:									
Full	205. 2	513. 0	5. 31	11. 16	.....	9. 77	84. 91	. 764	3. 80
Limited	207. 8	325. 0	5. 37	11. 17	.....	9. 82	84. 80	. 765	3. 79

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 8

Water allowance.	Total milk.	Total water.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
Full	252. 2	573. 0	4. 55	11. 48	1. 033	9. 30	86. 15	0. 730	3. 17
Do	253. 0	553. 0	4. 15	10. 50	1. 033	9. 10	86. 75	. 723	3. 12
Transition	234. 9	346. 0	4. 20	10. 46	1. 034	9. 39	86. 41	. 707	3. 20
Limited	220. 7	350. 0	4. 30	9. 49	1. 034	9. 44	86. 26	. 731	3. 14
Do	208. 0	350. 0	4. 65	9. 67	1. 034	9. 35	86. 00	. 714	3. 11
Transition	217. 6	509. 0	4. 30	9. 37	1. 033	8. 98	86. 72	. 727	3. 11
Full	205. 7	500. 0	4. 30	8. 85	1. 034	9. 25	86. 45	. 724	3. 06
Do	209. 4	536. 0	4. 50	9. 42	1. 033	9. 18	86. 32	. 732	3. 17
Average:									
Full	230. 1	540. 0	4. 37	10. 06	.....	9. 21	86. 88	. 727	3. 13
Limited	214. 3	350. 0	4. 47	9. 58	.....	9. 39	86. 13	. 722	3. 12
COW 17									
Full	194. 9	465. 0	5. 30	10. 33	1. 034	9. 73	84. 97	. 74	3. 70
Do	206. 8	437. 0	4. 95	10. 24	1. 035	9. 88	85. 17	. 72	3. 77
Transition	173. 5	267. 0	5. 28	9. 02	1. 035	9. 73	84. 99	. 715	3. 62
Limited	188. 2	310. 0	5. 15	9. 69	1. 034	9. 53	85. 32	. 720	3. 68
Do	174. 0	300. 0	5. 20	9. 05	1. 035	9. 92	84. 88	. 755	3. 68
Transition	184. 7	443. 0	5. 18	9. 57	1. 034	9. 44	85. 38	. 740	3. 81
Full	164. 9	499. 0	5. 20	8. 57	1. 033	9. 69	85. 11	. 770	3. 86
Do	156. 4	504. 0	5. 18	8. 10	1. 033	9. 70	85. 12	. 755	3. 76
Average:									
Full	180. 7	476. 0	5. 16	9. 31	.....	9. 75	85. 09	. 744	3. 77
Limited	181. 1	305. 0	5. 17	9. 37	.....	9. 72	85. 10	. 737	3. 68
COW 9									
Full	199. 7	410. 0	4. 40	8. 79	1. 031	8. 83	86. 77	. 744	2. 78
Do	193. 7	432. 0	4. 15	8. 04	1. 030	8. 40	87. 45	. 709	2. 65
Transition	181. 0	386. 0	4. 20	7. 60	1. 031	8. 53	87. 27	. 711	2. 76
Limited	163. 5	300. 0	4. 05	6. 62	1. 032	8. 79	87. 16	. 724	2. 72
Do	142. 9	300. 0	4. 15	5. 93	1. 031	8. 64	87. 21	. 703	2. 54
Transition	165. 7	526. 0	4. 10	6. 79	1. 030	8. 27	87. 63	. 698	2. 69
Full	170. 4	556. 0	4. 15	7. 07	1. 031	8. 53	87. 32	. 712	2. 73
Do	164. 9	541. 0	4. 30	7. 09	1. 031	8. 65	87. 05	. 704	2. 84
Average:									
Full	182. 2	485. 0	4. 25	7. 75	.....	8. 60	87. 15	. 717	2. 75
Limited	153. 2	300. 0	4. 10	5. 77	.....	8. 71	87. 18	. 713	2. 63
COW 14									
Full	269. 1	429. 0	5. 10	13. 72	1. 033	9. 17	85. 73	. 723	3. 04
Do	263. 9	470. 0	4. 80	12. 67	1. 032	9. 11	86. 09	. 723	3. 10
Transition	236. 5	338. 0	5. 40	12. 77	1. 033	9. 10	85. 50	. 754	3. 18
Limited	232. 9	305. 0	5. 00	11. 64	1. 033	8. 97	86. 03	. 756	3. 22
Do	222. 6	300. 0	5. 10	11. 36	1. 033	8. 91	85. 99	. 739	3. 25
Transition	235. 2	566. 0	4. 90	11. 52	1. 032	8. 84	86. 26	. 727	3. 16
Full	227. 6	494. 0	5. 05	11. 50	1. 032	9. 06	85. 89	. 737	3. 21
Do	211. 1	484. 0	4. 70	9. 92	1. 031	8. 99	86. 31	. 724	3. 06
Average:									
Full	242. 9	469. 0	4. 91	11. 95	.....	9. 09	86. 00	. 727	3. 10
Limited	227. 7	302. 0	5. 05	11. 50	.....	8. 94	86. 51	. 747	3. 23

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 2

Water allowance.	Total milk.	Total water.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
Full.....	238. 6	517. 0	5. 60	13. 37	1. 033	9. 55	84. 85	0. 724	3. 50
Do.....	236. 5	595. 0	4. 85	11. 47	1. 034	9. 68	85. 47	. 737	3. 67
Transition....	201. 6	388. 0	5. 60	11. 29	1. 035	9. 92	84. 48	. 775	3. 85
Limited.....	181. 9	350. 0	5. 50	10. 00	1. 035	9. 57	84. 93	. 769	3. 82
Do.....	188. 5	350. 0	5. 55	10. 47	1. 035	9. 57	84. 88	. 746	3. 77
Transition....	172. 7	589. 0	5. 30	9. 15	1. 032	9. 32	85. 38	. 738	3. 48
Full.....	189. 9	630. 0	4. 95	9. 40	1. 034	9. 54	85. 51	. 754	3. 56
Do.....	202. 9	639. 0	4. 70	9. 54	1. 033	9. 62	85. 68	. 747	3. 89
Average:									
Full.....	217. 0	595. 0	5. 02	10. 94	.....	9. 60	85. 38	. 740	3. 65
Limited..	185. 2	350. 0	5. 52	10. 23	.....	9. 57	84. 90	. 757	3. 79

In studying the data obtained in these trials it will be noted that all the milk constituents except the fat show very little variation during the different periods, and that these differences are attributable more to the individual animals than to the character of the ration. Taking the average figures for the two classes of rations, it will be seen that the full water allowance ration tended to increase the quantity of milk produced and to cause a slight reduction of the fat content of the milk. A study of the data for individual cows by separate periods, however, will show that this average effect of the different rations is caused more by the order in which the rations are fed than by their character. Of the data obtained from the eight cows used in this test those from only one (No. 2) show indication of any effect of the ration upon the composition of the milk, and the data from the seven other cows are so negative that this variation is probably caused more by the individual than by the ration. Two of the cows, Nos. 17 and 19, show practically no variation in either quantity or quality of the milk produced; one other, No. 100, decreased gradually in the quantity of milk produced and increased gradually in quality, regardless of the ration; while the remaining four, Nos. 8, 9, 14, and 21, gave milk the fat content of which varied considerably from normal in different periods, even on the same ration. These variations were independent of the character of the ration—that is, the abnormal percentage of fat was in some cases found when the full allowance of water was given and in other cases when the quantity was reduced. A summing up of all the data obtained shows that the feeding of rations whose water content is varied by controlling the quantity of water drunk has no influence upon the composition of the milk produced.

TURNIPS VERSUS DRY-ROUGHAGE RATION

In this series of experiments four cows were used, the experimental period consisting of six test periods and two transition periods. Figure 1 shows the grouping of the cows and the character of the ration fed during each period.

As much as 90 pounds of turnips a day was fed to the cows on the wet-roughage ration, with the addition of 4 pounds of clover hay. The roughage ration of the dry-roughage group consisted entirely of clover hay. The grain ration was the same for both groups. In Table II

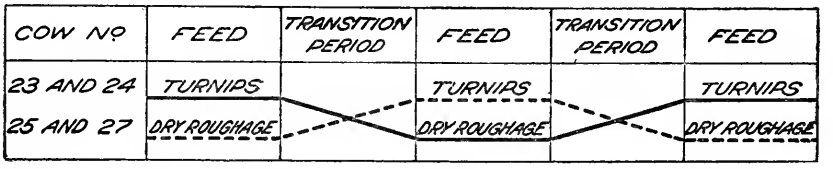


FIG. 1.—Grouping of cows and kind of ration fed cows 23, 24, 25, and 27

both the quantity of water drunk and the total water content of the turnips are given, turnips being considered as having 90 per cent of water, as shown by Henry and Morrison (5, p. 645).

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*

COW 23

Ration.	Total milk.	Water in ration.	Turnips.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*P. ct.*	*Lb.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Wet	234.7	94	774	4.10	9.62	1.032	8.64	87.26	0.750	3.18
Do	236.9	123	810	4.30	10.19	1.030	8.50	87.20	.720	3.09
Transition	225.2	446	261	4.03	9.08	1.031	8.52	87.45	.735	3.17
Dry	212.4	712	.....	4.00	8.50	1.030	8.44	87.56	.715	3.09
Do	214.6	734	.....	4.03	8.65	1.030	8.40	87.57	.700	3.13
Transition	204.2	190	630	3.90	7.96	1.030	8.55	87.55	.725	3.11
Wet	198.6	62	810	4.13	8.20	1.030	8.47	87.40	.725	3.24
Do	192.9	88	810	4.05	7.81	1.031	8.69	87.26	.740	3.28
Average:										
Wet	215.8	92	801	4.14	8.95	.....	8.57	87.28	.732	3.20
Dry	213.5	723	.....	4.01	8.57	.....	8.42	87.56	.707	3.11

COW 24

Wet	255.1	84	774	4.10	10.50	1.035	9.56	86.34	.710	3.50
Do	251.3	72	810	4.10	10.30	1.035	9.73	86.17	.690	3.55
Transition	234.5	389	261	4.30	10.08	1.035	9.48	86.22	.690	3.51
Dry	226.4	607	.....	3.80	8.60	1.034	9.32	86.88	.645	3.37
Do	226.7	658	.....	4.00	9.07	1.033	9.21	86.79	.635	3.47
Transition	234.0	119	630	3.81	8.92	1.033	9.44	86.75	.695	3.44
Wet	237.2	56	810	4.10	9.73	1.033	9.31	86.59	.680	3.51
Do	227.1	144	810	4.10	9.31	1.034	9.51	86.39	.715	3.64
Average:										
Wet	242.7	89	801	4.10	9.96	.....	9.53	86.37	.699	3.52
Dry	226.5	632	.....	3.90	8.83	.....	9.26	86.83	.640	3.42

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*—Continued

COW 25

Ration.	Total milk.	Water in ration.	Turnips.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*P. ct.*	*Lb.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Dry	199. 8	604	.....	4. 23	8. 45	1. 033	9. 11	86. 66	. 750	3. 14
Do	195. 5	541	.....	4. 30	8. 41	1. 033	9. 07	86. 63	. 730	3. 11
Transition	218. 2	158	85	4. 25	9. 27	1. 032	8. 89	86. 86	. 745	3. 21
Wet	203. 0	.....	810	3. 98	8. 08	1. 032	9. 03	86. 99	. 715	3. 34
Do	198. 0	.....	810	4. 00	7. 92	1. 033	9. 10	86. 90	. 725	3. 40
Transition	177. 3	304	180	4. 23	7. 50	1. 032	9. 17	86. 60	. 755	3. 49
Dry	175. 3	505	.....	4. 50	7. 89	1. 032	8. 81	86. 69	. 715	3. 18
Do	160. 4	488	.....	4. 55	7. 30	1. 031	8. 87	86. 58	. 730	3. 28
Average:										
Dry	182. 7	532	.....	4. 39	8. 01	.....	9. 01	86. 64	. 731	3. 18
Wet	200. 0	.....	810	3. 99	8. 00	.....	9. 06	86. 94	. 720	3. 37

COW 27

Dry	223. 2	591	.....	4. 30	9. 60	1. 033	9. 04	86. 66	. 730	3. 13
Do	207. 7	578	.....	4. 10	8. 52	1. 033	9. 18	86. 72	. 710	3. 12
Transition	223. 3	132	585	4. 20	9. 38	1. 032	8. 90	86. 89	. 735	3. 14
Wet	237. 8	.....	810	4. 00	9. 51	1. 033	8. 81	87. 19	. 695	2. 99
Do	240. 0	.....	810	4. 04	9. 70	1. 033	9. 02	86. 94	. 705	3. 29
Transition	214. 0	407	180	4. 03	8. 62	1. 034	9. 09	86. 88	. 745	3. 24
Dry	199. 0	548	.....	4. 05	8. 06	1. 032	9. 01	86. 94	. 750	3. 28
Do	175. 4	588	.....	4. 30	7. 54	1. 031	8. 82	86. 88	. 765	3. 15
Average:										
Dry	201. 3	576	.....	4. 19	8. 43	.....	9. 01	86. 80	. 741	3. 17
Wet	238. 9	.....	810	4. 02	9. 60	.....	8. 91	87. 06	. 730	3. 14

In this series of experiments the data show conflicting results. All the cows gave more milk when fed the turnip ration, and they also ate that ration much more readily than they did the entire dry-roughage one. The two cows that were fed the ration in the order wet-dry-wet gave milk of a higher fat content on the wet ration, while those fed in the dry-wet-dry order gave the higher percentage of fat when the dry ration alone was fed. None of the other constituents of the milk were appreciably affected, and in the case of the fat content the data are so conflicting that they seem to have been caused by some factor other than the ration.

DRY VERSUS WET BEET PULP

Two cows were used in this trial, one being fed wet, dry, and wet beet pulp in successive periods, with a transition period after each change in ration, and the ration of the second cow being just the reverse. While being fed dry beet pulp each cow received 10 pounds daily. The wet ration consisted of 40 pounds of the wet beet pulp, or 10 pounds of the

dry, with 30 pounds of water added, the pulp used having been found to absorb three times its weight of water. In all conditions except as to the pulp the two rations were alike for each cow in the different periods. In Table III the quantity of water in the beet pulp, as well as the quantity of water drunk, is given:

TABLE III.—*Comparison of the effect of dry beet pulp and wet beet pulp on the composition of milk*

COW 22

Ration.	Total milk.	Water in ration.	Pulp.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*Per ct.*	*Lb.*		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Dry	209. 6	590	.....	4. 80	10. 06	1. 034	9. 93	85. 27	0. 769	3. 65
Do	201. 8	540	.....	4. 85	9. 79	1. 036	10. 08	85. 07	. 797	3. 70
Wet	199. 3	273	300	4. 65	9. 27	1. 035	10. 00	85. 35	. 789	3. 89
Do	189. 5	306	300	4. 65	8. 81	1. 035	10. 15	85. 20	. 796	3. 88
Transition	185. 4	487	49	4. 80	8. 90	1. 035	9. 85	85. 35	. 795	3. 92
Dry	180. 9	479	.....	4. 80	8. 68	1. 036	9. 76	85. 44	. 797	3. 88
Do	167. 7	472	.....	4. 90	8. 22	1. 035	9. 59	85. 51	. 790	3. 82
Average:										
Dry	190. 0	520	.....	4. 84	9. 19	.....	9. 84	85. 32	. 788	3. 76
Wet	194. 4	290	300	4. 65	9. 04	.....	10. 07	85. 27	. 792	3. 88

COW 18

Wet	193. 7	340	300	5. 10	9. 88	1. 032	9. 23	85. 67	. 740	3. 11
Do	185. 0	369	300	5. 20	9. 62	1. 033	9. 19	85. 61	. 747	3. 30
Dry	196. 6	472	.....	5. 00	9. 83	1. 034	9. 60	85. 40	. 730	3. 51
Do	176. 9	511	.....	5. 40	9. 55	1. 033	9. 48	85. 12	. 733	3. 41
Transition	183. 2	348	228	5. 20	9. 53	1. 034	9. 45	85. 35	. 760	3. 62
Wet	166. 9	327	300	5. 60	9. 35	1. 035	9. 40	85. 00	. 766	3. 64
Do	159. 6	383	300	5. 60	8. 94	1. 034	9. 30	85. 10	. 754	3. 65
Average:										
Wet	176. 3	355	300	5. 37	9. 45	.....	9. 28	85. 34	. 752	3. 42
Dry	186. 7	492	.....	5. 20	9. 69	.....	9. 54	85. 27	. 731	3. 46

The data from these two cows give negative results so far as the effect of the water in the ration upon the composition of the milk is concerned. One cow, No. 22, gave milk slightly lower in fat content when the wet beet pulp was fed; but the other gave opposite results, the milk testing higher than that produced when the preceding dry ration was fed. The quantity of milk produced by both cows decreased at a normal rate.

GREEN VERSUS CURED CRIMSON CLOVER

In this series of experiments four cows were used. For a period of 10 days they were each fed all the fresh-cut green crimson clover that they would consume, and composite samples were taken during the period.

Later, when the clover had been harvested and had become well cured, the same four cows were fed all the cured product that they would consume, and composite samples again taken. No weights of water drunk were taken, but as the green clover contained 71.23 per cent of water and the cured hay but 8.33 per cent, there was an appreciable difference in the quantity of water in the rations of the two test periods. Table IV gives the results for each cow. The figures in parentheses following the class of ration show the total number of pounds of the cured or green clover fed.

TABLE IV.—*Comparison of the effect of green and cured crimson clover on the composition of milk*

Ration.	Milk.	Total water in roughage.	Fat.		Specific gravity.	Moisture.	Ash.	Total protein.
					COW 23			
	Lb.	*Lb.*	*Per ct.*	*Lb.*		*Per ct.*	*Per ct.*	*Per ct.*
Green (405)	132.0	288	5.81	4.40	1.029	86.94	0.723	3.18
Cured (180)	107.1	15	4.53	4.23	1.031	86.97	.744	3.38
					COW 25			
Green (415)	163.2	296	4.05	6.61	1.030	87.26	.724	3.17
Cured (180)	167.3	15	3.60	6.02	1.032	87.45	.742	3.19
					COW 27			
Green (400)	161.1	285	3.75	6.04	1.030	87.58	.738	3.05
Cured (165)	128.0	14	3.60	4.61	1.032	87.35	.783	3.17
					COW 201			
Green (505)	333.5	360	3.65	12.17	1.028	88.36	.696	2.78
Cured (220)	297.2	18	3.20	9.51	1.030	88.85	.725	2.77

The length of time covered by this series of experiments, 10 days on each ration, was too short to give more than an indication of the results which a complete investigation would give. The data obtained, however, show that the water in the ration supplied by a green roughage, as compared with the cured product, does not lower the fat content of the milk. The results of these experiments would even indicate an opposite effect, for in all cases the cows gave higher testing milk and three of them produced more milk on the green feed.

SUMMARY

Four different methods of varying the water content of the ration were used in this work.

(1) A full versus a limited allowance of drinking water.
(2) Turnips versus a dry-roughage ration.
(3) Wet versus dry beet pulp.
(4) Green versus dry crimson clover.

Eight cows were used in the experiments conducted by the first method, four in the second, two in the third, and four in the fourth.

In every case except when the crimson clover was fed the amount of water drunk by the different animals, as well as the difference in the water content of the roughages under comparison, was determined.

With all except one cow, No. 22 in the wet versus dry beet-pulp group, the amount of water in the dry ration did not exceed 75 per cent of that supplied by the wet ration, and with some cows that were given a limited allowance of water the dry ration contained less than 60 per cent of the water content of the full-allowance ration.

Cow 22 in the wet versus dry beet-pulp group received, when the dry ration was fed, 88 per cent of the water content of the wet ration.

In the green versus cured crimson-clover group, the former contained 71.23 per cent water and the latter 8.33 per cent. The daily ration of green clover varied from 40 to 50 pounds per head, and of the cured hay from 16 to 22 pounds per head.

Certain individual cows at times produced milk having an abnormal fat content. This effect was apparently independent of the ration, as it occurred not only with the high water-content ration but with the dry as well. A study of the data obtained in the four series shows that the watery character of the ration has no effect upon the fat content of the milk.

There was even less variation in the other milk constituents than in the fat. This indicates that rations of varying water content have no effect upon the composition of milk.

LITERATURE CITED

(1) GILCHRIST, D. A.
1909. Variations in the composition of milk and their probable causes. *In* Durham Co. Coun. Educ. Com. Rpts. Dairy Invest. Offerton Hall, 1909, p. 7–27.

(2) GREAT BRITAIN BOARD OF AGRICULTURE.
1911. Effect on milk of water or watery food given to cows. *In* Jour. Bd. Agr. [London], v. 17, no. 11, p. 910–911.

(3) ———
1913. The effect of watery foods on milk. *In* Jour. Bd. Agr. [London], v. 20, no. 5, p. 385–392.

(4) HANSSON, Nils.
1912. Der verschiedenartige Einfluss der Futtermittel auf die Milchabsonderung und Fettproduktion der Kühe. *In* Fühling's Landw. Ztg., Jahrg. 61, Heft 10, p. 337–351.

(5) HENRY, W. A., and MORRISON, F. B.
1915. Feeds and Feeding . . . ed. 15, 691 p. Madison, Wis.

(6) HOLTSMARK, B.
1897. Nogle Iagttagelser over Melkens Fedtinhold ved Turnipsfodring. *In* Tidsskr. Norske Landbr., Aarg. 4, p. 161–169.

(7) JONES, C. B.
1907. The effect of brewers' grain on milk. *In* Durham Co. Coun. Educ. Com. Offerton Bul. 2, p. 23–36. *Also in* Durham Co. Coun. Educ. Com. Rpts. Dairy Invest. Offerton Hall, p. 81–92. 1909.

(8) JORDAN, W. H.
1908. Investigations in animal nutrition. *In* 26th Ann. Rpt. N. Y. State Agr. Exp. Sta., pt. 3 (25th Anniv. Rpt. 1882/1907), p. 66–71.

(9) KOCH, B.
1901. Untersuchungen über den Einfluss der Menge des aufgenommenen Wassers auf die Milchsekretion des Rindes. *In* Jour. Landw., Bd. 49, Heft 1, p. 61–88.

(10) LAUDER, Alexander, and FAGAN, T. W.
1912. On the effect of heavy root feeding on the yield and composition of milk. Edinb. and East of Scot. Col. Agr. Rpt. 26, 56 p., tab.

(11) MCCONNELL, Primrose.
1911. Watering milk. *In* Dairy, v. 23, no. 268, p. 102.

(12) TANGL, F., and ZAITSCHEK, A.
1911. Über den Einfluss verschiedener wässiger Futtermittel auf die Menge und Zusammensetzung der Milch. *In* Landw. Vers. Stat., Bd. 74, Heft 3/5, p. 183–249.

(13) WALKER, F. P.
1909. A second experiment to test the effect of brewers' grains on the quantity and quality of milk. *In* Durham Co. Coun. Educ. Com. Offerton Bul. 3, p. 5–20. *Also in* Durham Co. Coun. Educ. Com. Rpts. Dairy Invest. Offerton Hall, p. 93–107. 1909.

CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS

[PRELIMINARY PAPER]

By ERWIN F. SMITH,

Pathologist in Charge, Laboratory of Plant Pathology, Bureau of Plant Industry

Some recent experiments with crowngall have led to a number of discoveries which may be summarized as follows:

First, as everyone knows, the tendency of cambium is not simply to go on indefinitely producing more cambium but to elaborate out of its embryonic elements formed structures, tracheids, wood vessels, wood fibers, ray cells, sieve tubes, etc., all having a definite arrangement and a well-defined polarity, but when internodal stem cambium is inoculated with the crowngall organism (*Bacterium tumefaciens*) the ordinary physiological tendencies are upset, as already shown in 1911 and 1912,[1] and several very interesting new phenomena make their appearance: (1) The elements of the formed or mature tissues are produced in less numbers than ordinarily, and these elements have lost the whole or a considerable part of their polarity, so that the most bizarre complexes of twisted and distorted tissues arise; (2) the parenchymatous elements are greatly increased in number and reduced in size, since under the bacterial stimulus many of the cambium cells appear to have lost all power to produce mature tissues and at the same time have acquired a new growth impetus, a tendency to an uncontrolled, pathologically embryonic, cell multiplication, the result of which is a tumor of indefinite extension—the ordinary naked crowngall, containing the distorted formed elements above referred to and in addition exhibiting a marked hyperplasia of the parenchyma; (3) correlative with these changes, over which the plant has no control, is a tendency to open wounds and to early decay and also to the formation of daughter tumors.

Second, when, by means of very shallow needle pricks, similar inoculations are made into the internodal cortex of young stems (the so-called fundamental tissue, which is still in a growing condition) a similar cell proliferation occurs, the elements of which are very small in comparison with those from which they have developed, because under the changed stimulus they are kept embryonic and are compelled to divide soon after previous divisions, so that they can never reach maturity either in size

[1] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Plant Indus. Bul. 213, 215 p., 36 pl. 1911.

Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crown-gall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, 60 p., 109 pl. 1912.

Journal of Agricultural Research, Vol. VI, No. 4
Dept. of Agriculture, Washington, D. C. Apr. 24, 1916
ds G—77

or function as long as the stimulus lasts. These inoculations (on the Paris daisy) have brought out another interesting fact. As the tendency of young fundamental tissue (the growing point) is to form a stele in its center, so when the mature tissues of the stem cortex are brought under the new stimulus and begin to proliferate, in the manner of embryonic tissues, primitive but imperfect stele-forming tendencies are developed in the tumor. I have not seen an actual shoot produced by such a tumor; but sieve tubes and trachei are formed in it (out of descendants of cortex cells, be it remembered); and cross sections of some of these small tumors show that these stelar elements have a tendency to be arranged in the form of a closed structure (primitive stele), although often this is not pronounced. These superficial tumors have no connection with the xylem or phloem of the true stele, for in no case did the needle punctures enter as far as the phloem, much less the cambium, and serial sections show clearly that all of their structures (blastomous cells, trachei, and sieve tubes) have been developed wholly, out of cortex cells (probably cortex mother cells). After a few weeks such shallow tumors cease to grow, or develop very slowly, owing to imperfect nutrition (lack of all connection with the xylem and phloem of the plant).

Third, when the crowngall organism (hop strain) is inoculated into the leaf axils of young growing plants (species of Pelargonium, Nicotiana, Lycopersicum, Citrus, Ricinus, etc.) the buds of which are in a dormant state and which under ordinary conditions will continue dormant—namely, unless the top of the plant is removed—a new type of tumor develops, one hitherto not seen in crowngall. Inoculating in this way I have obtained tumors covered all over with diminutive, abortive leafy shoots, or flower shoots, if flower anlage have been disturbed. The shoots may be variously twisted, fused, and fasciated, as in the common house geranium (*Pelargonium* spp.) shown in Plate XVIII. This apparently is what happens: The growth of the tumor distorts the tissues, tearing the anlage into small fragments which are variously distributed and develop on or in the tumor into organs of a size proportional to the size of the included fragment—here as part of an ovary or anther, there as a shoot. These pathological shoots live but a short time and are quite unable to carry on the normal activities of the plant when the other leaves are removed. I have believed for a long time that fasciation must be due to a bacterial infection; but this is, I believe, the first time that anyone has obtained it by means of a pure-culture inoculation.

The results obtained by inoculating the upper leaf axils of young growing plants of the castor-oil plant (*Ricinus communis*) are prompt and quite as striking (Pl. XIX).

On tobacco plants (*Nicotiana tabacum*) these teratoid tumors, developed in leaf axils (Pls. XX and XXII), have also produced secondary tumors repeating the structure of the parent tumor. Such tumors have been obtained both in stems and leaves, especially when inoculations were

made early; and they contain, along with the proliferating tumor cells (blastomous cells), the same teratoid elements as the primary tumor. These are true daughter tumors, being connected back to the primary tumor by a tumor strand which is quite different both in structure and in location (Pl. XXI) from that occurring in the Paris daisy. The latter, it will be remembered, follows the line of the spiral vessels in the inner wood, and is parenchymatous in its structure, containing only here and there some vessels (scattered trachei). This tobacco tumor strand occurs in the cortex, consists almost entirely of vessels, and is a true stem (stele), although developed under a pathological stimulus, and in a part of the plant where no stele was ever seen before—namely, in the outer cortex, through which it can be traced (parallel to the long axis of the stem) for long distances and from which at intervals leafy tumors are sent to the surface of the plant. From its frequent proliferation in the form of tumors it is evident that parenchymatous (blastomous) elements must also occur in the strand, but they are not abundant. In fact, in the parts I have examined they are almost as infrequent as are trachei in the daisy strand. Cross sections and longitudinal sections of this remarkable tumor strand show it to have spiral vessels in its center, surrounded by trachei cut by ray cells, beyond which is a cylinder of cambium surrounded by a cylinder of phloem, containing well-developed sieve tubes. This tiny stele has no cortex or epidermis because it does not need any, being surrounded and sufficiently protected by the normal cortex of the tobacco stem. This is a phenomenon due apparently to my new manner of inoculation (into shoot anlage), because some years ago by inoculating internodally on tobacco stems I obtained and figured[1] tumors and a tumor strand in cortex corresponding to those found in the Paris daisy—that is, composed chiefly of small-celled parenchyma. The difference in results must therefore be due to difference in the kind of tissue inoculated, each developing pathologically according to its own growth tendencies.

Fourth, on some plants (which were tobaccos) I have also obtained leafy tumors by making my bacterial inoculations *in places where no bud anlage are known to exist*—for example, in the middle of leaves. Ordinarily when leaf tissue in tobacco grows, it only produces more leaf tissue;[2] but when the crowngall organism (hop strain) is pricked into midribs or side veins, tumors arise and a portion of them are leafy—that is, bear shoots. I have obtained 27 such leafy tumors on a single plant and several on a single leaf, all within a period of a few weeks (Pl. XXIII). It is easy to obtain them. The young leaves yield a larger proportion of such tumors than the older ones, and I have observed no shoot-bearing tumors on leaves which were fairly well developed when inoculated.

[1] Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crown-gall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, pl. 102–103. 1912.

[2] I have never got any leaf cuttings of it to take root.

Rapidly developing young tissues seem to be necessary. Here again, a changed stimulus has produced a more embryonic and primitive condition, as shown by the appearance of these shoots. It is a pathological phenomenon, but one of more than passing interest, for, unless I am much mistaken, it has wide physiological and pathological bearings. It is another proof that the immature cell wherever it is located carries the inheritance of the whole organism, and that what it will finally become, as it matures, depends on the stimuli withheld from it or applied to it. In other words, it is so much evidence that any young cell may become a totipotent cell if it is subjected to the proper stimulus, and this stimulus may be either *physiological*, resulting in a normal structure, as when the top of a plant is removed and a new top grows in its place out of so-called adventitious buds (regeneration phenomena), or *pathological*, resulting in an embryonic teratoma, as when a tumor-producing schizomycete is introduced into sensitive growing tissues.

PLATE XVIII

Teratoid crowngalls produced in *Pelargonium* spp. by inoculating *Bacterium tumefaciens* (hop organism through sunflower) into upper leaf axils on January 13, 1916. Photographed at the end of 74 days. At *X* the top of the shoot bearing five or six leaves was removed to show the tumor more distinctly. All of the leafy shoots here shown and many others too small to be seen distinctly in the photograph are outgrowths from the tumor, which also bears red abortive flower anlage. The upper shoot (*L*) was also flattened and fasciated (several shoots fused together) and the front leaves (*P P*) were turning yellow and dying.


L
P
P
X

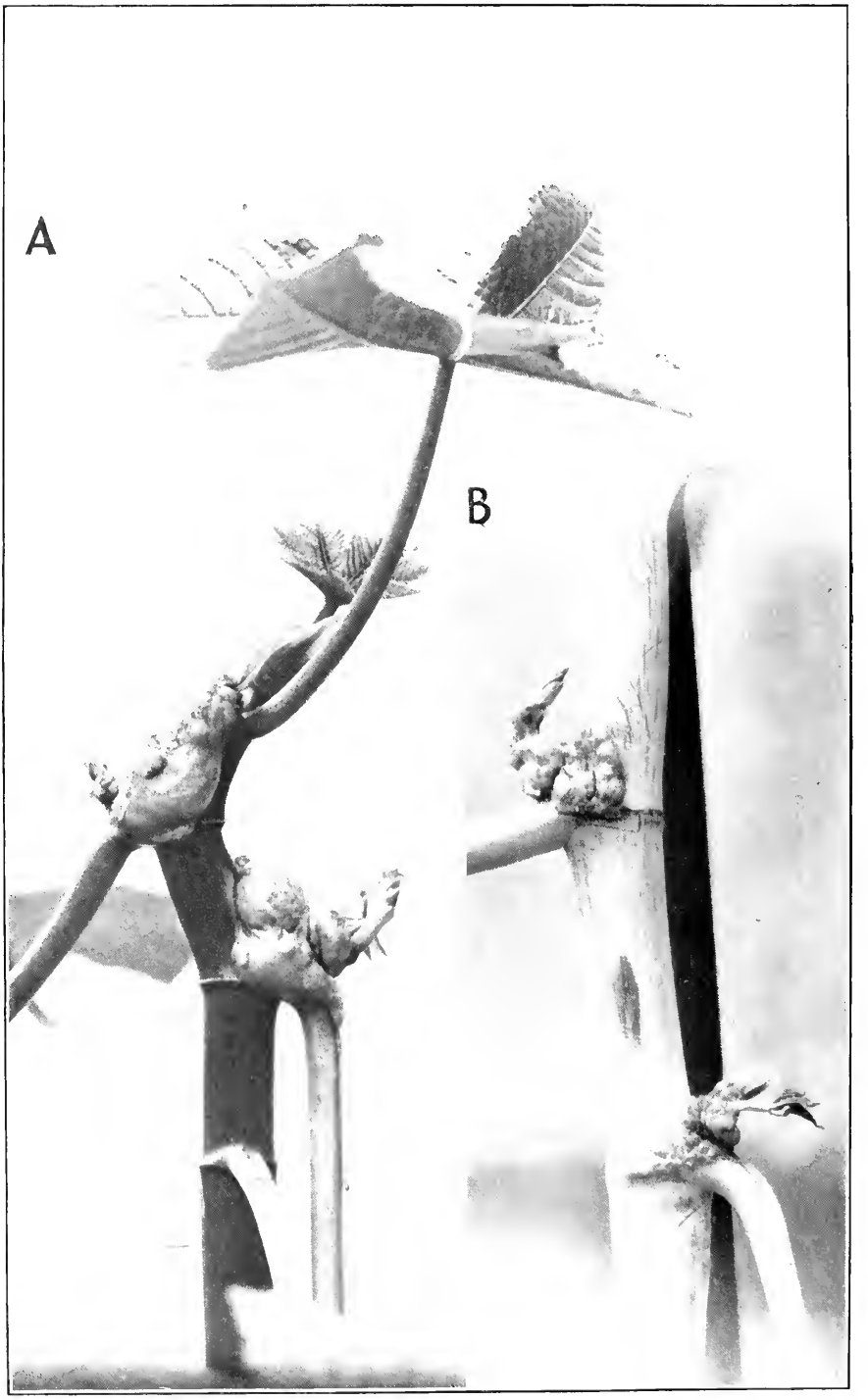

A
B

PLATE XIX

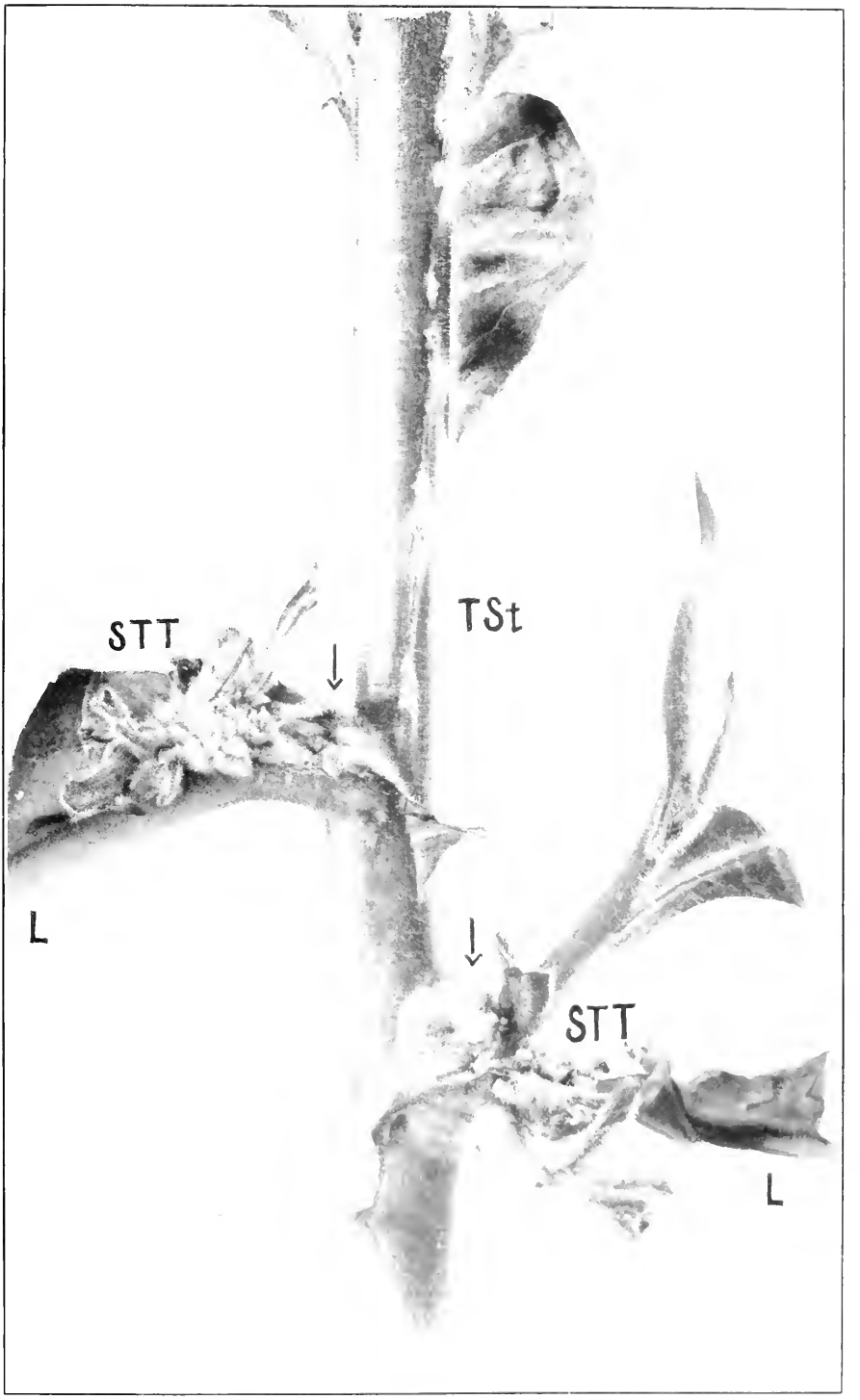
Teratoid crowngalls produced in castor-oil plant (*Ricinus communis*) by inoculating *Bacterium tumefaciens* (hop strain), the inoculations being made in the upper leaf axils of young, vigorous, unbranched plants.

Fig. A.—A red-stem variety. Leaves reflexed; axis distorted; and feeble shoots developing out of the axillary tumors. There are on the tumors other smaller shoots not shown here distinctly. Time, 13 days.

Fig. B.—A green-stem glaucous variety. As in figure A, but time 17 days. Here also internal growths (root anlage) are pushing up the tissues of the stem below the lower leaf. A few days later these roots appeared on the surface, both of this internode and of the one above it. This phenomenon has been recorded previously by the writer as sometimes occurring on inoculated stems of the Paris daisy and other plants in the vicinity of developing tumors (Smith, E. F. Bacteria in Relation to Plant Diseases. vol. 2, fig. 26. 1911).

PLATE XX

Teratoid crowngalls produced in tobacco by inoculating *Bacterium tumefaciens* (isolated from a hop tumor several years ago and passed through a sunflower in 1915). The inoculations were made by needle pricks in the axils of the lower leaves (under the arrows), at which places small leafy tumors developed. These sent tumor strands into the midribs of both leaves (*L L*) and later secondary teratoid tumors (*S T T*) burst through and covered the top of the midrib. From the upper leaf axil also a tumor strand developed, passing upward through 5 internodes and then out into the midrib of a leaf for several inches, giving rise at frequent intervals to tumors bearing leafy shoots (teratoid elements) and to others free from them. This tumor strand (*T St*) was not on the surface of the stem, as might appear from the photograph, but was near enough to show through as a translucent band about 2 mm. wide. Time, 26 days.


STT
TSt
L
STT
L

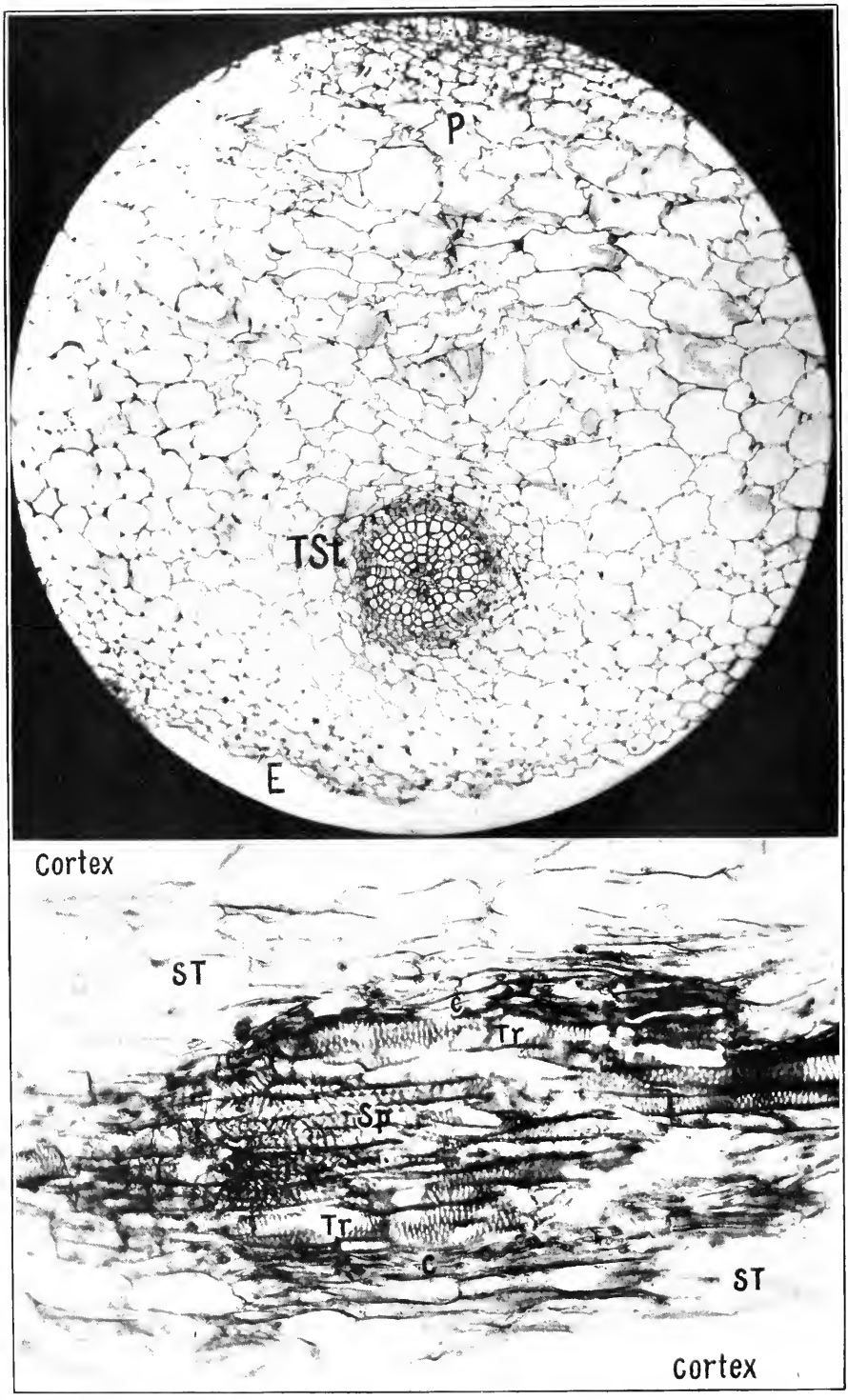

P
TSt
E
Cortex
ST
C
Tr
Sp
Tr
C
ST
Cortex

PLATE XXI

The teratoid tumor strand of Plate XX, which gives rise during its course to more than 30 small tumors.

Top. Cross section of outer part of right side of stem of tobacco plant shown on Plate XX. *P*, outer edge of the phloem; *E*, epidermis; *T St*, tumor strand, which is bedded in the normal cortex of the stem.

Bottom. Longitudinal section from upper part of the above tumor strand, more highly magnified, showing it to be a true stele. The coarse-celled tissue at top and bottom is the normal cortex of the stem. The pathological tissues are *S T*, sieve tubes; *C*, cambium; *Tr*, trachei; *Sp*, spiral vessels.

PLATE XXII

Teratoid crowngalls produced in a tobacco plant by inoculating *Bacterium tumefaciens* (hop strain through sunflower) into the leaf axils. Small tumors soon appeared where inoculated and these are now covered with pale leafy shoots which have swollen (tumefied) bases and are beginning to die. The top was cut away on the 26th day, and the plant was unable to make a new one out of these pathological shoots, but has grown it (*X*) from an uninoculated lower leaf axil. Time, 73 days.


X


A
C
B

PLATE XXIII

Teratoid crowngalls produced in tobacco leaves with the hop strain of *Bacterium tumefaciens* by local (leaf) inoculations—that is, inoculation in places where shoot anlage are not known to exist.

Fig. A.—Portion of an upper leaf showing four shoot-bearing tumors growing from upper surface of the inoculated midrib. Leaf inoculated February 16, 1916. Photographed on April 1.

Fig. B.—Same as *A*, but the leaf reversed and the midrib stripped of its blade to show two other shoot-bearing tumors which have developed from its under surface. Actual height of the tallest shoot, 1.5 cm.

Fig. C.—From middle of another leaf on the same plant as *A*, but further magnified and photo made on an orthochromatic plate to show the pale green character of the shoot as contrasted with the dark green of the surrounding leaf (which is also in shadow). This tumor and its shoot arise from a branch of the midrib, the latter in cross section being shown at *X*. A smaller teratoid tumor bearing two shoots (at either side of *C*) developed on the upper surface of the leaf and the one bearing the longer shoot on its lower surface. The actual length of this shoot was 1.5 cm. The leaf was curved downward and the shoot was growing out horizontally. Time, 45 days.

27472°—16——4

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
15 CENTS PER COPY
SUBSCRIPTION PRICE, $3.00 PER YEAR

△

Vol. VI MAY 1, 1916 No. 5

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE

WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., MAY 1, 1916 NO. 5

EFFECT OF CERTAIN SPECIES OF FUSARIUM ON THE COMPOSITION OF THE POTATO TUBER[1]

By LON A. HAWKINS,
Plant Physiologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry

INTRODUCTION

Potato tubers (*Solanum tuberosum*) are subject to attack by various parasitic fungi. Some of these organisms invade the tuber, kill the cells, break down the cell walls, and cause, directly or indirectly, a more or less complete disorganization of the host tissue. What constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or to build up its own tissue are of considerable interest in the study of the physiology of parasitism. It was to obtain information on the effect of some potato tuber rot fungi upon the tissues of the host plant that the present study was planned and carried out. In this investigation the effect of *Fusarium oxysporum* Schlecht. and *F. radicicola* Wollenw. on the sucrose, reducing-sugar, starch, pentosan, galactan, and crude-fiber content of the potato was studied. Some experiments were duplicated also with *F. coeruleum* (Lib.) Sacc.

The three species of Fusarium just mentioned are all parasites on the potato tuber. Smith and Swingle (9)[2] considered *F. oxysporum* to be the cause of a serious rot of potato tubers. Wollenweber (10) did not agree with these writers, and contended that this fungus, while the cause of a wilt disease of the potato plant, was not able to rot the tubers. This conclusion of Wollenweber's has recently been disproved by Carpenter (4), who corroborates the findings of Smith and Swingle on this point. With this species and with *F. radicicola*, the latter considered by Wollenweber and by Carpenter to be the cause of a tuber-rot of considerable importance, the writer experienced no difficulty in obtaining well-rotted tubers in two to three weeks after inoculation.

[1] The work described in this paper was carried out in cooperation with the Office of Cotton and Truck-Crop Diseases. The writer thanks Mr. C. W. Carpenter, of that office, for cultures of the fungi used.
The writer's thanks are also due Mr. A. A. Riley, of the Office of Plant Physiological and Fermentation Investigations, for assistance in the experimental part of this study.
[2] Reference is made by number to "Literature cited," p. 196.

EXPERIMENTAL METHODS

As the methods for sterilizing, sampling, and inoculating followed in this study were similar to those outlined in a study of the brownrot of the peach (7), they will be discussed here only briefly. The sterile tubers were sliced longitudinally into four parts with a flamed knife. Particular attention was given to obtaining portions of approximately the same weight and same proportionate amount of cortex and pulp. Each quarter was placed in a small wide-mouthed flask or large test tube which had been stoppered with cotton, sterilized, and weighed. The containers with the portions of potatoes were weighed again and the samples were ready for inoculation. Two of the quarters from each potato were inoculated from stock cultures of some one of the fungi used in these experiments and a small quantity of sterile water was added to each of the four containers. The four samples, two inoculated and the two corresponding control samples, were placed side by side at room temperature until the inoculated portions were well rotted. They were then prepared and analyzed. The difference between the sound and the rotted portions in the content of the compounds determined was considered to be due to the action of the fungus. All control portions infected at the time the samples were prepared for analysis and all inoculated portions infected with organisms other than those used in the inoculations were discarded.

All samples were prepared for analysis by cutting them into very thin slices with a sharp knife and washing them into the proper vessel. Precautions were observed, of course, that none of the juice or pulp should be lost. The methods of analysis for agricultural chemists [1] were usually followed in the determination of the various compounds. The sugars were extracted from the tissue with alcohol and determined as in the work on the brownrot of the peach. The method of extraction is the alcohol method of Bryan, Given, and Straughn (3), somewhat modified to suit the conditions of the experiment. The amount of cane sugar was in all cases calculated from the reducing power of the extract before and after inversion with acid.

The starch determinations in the preliminary experiments were made only by the direct acid-hydrolysis method using the finely ground potato which had been extracted with alcohol. In the work with the sound and the rotted portions of the tubers, series of analyses were also made by the diastase method with subsequent acid hydrolysis.[1] Tollen's phloroglucid method [1] was followed in all cases in the determination of the pentosans. The methyl pentosans were determined according to the method of Ellett and Tollens (6), by extracting the precipitated phloroglucid with alcohol. The galactans and the crude fiber were de-

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

termined by the usual methods[1] in dry ether-extracted samples which had been ground. For the percentage of dry matter the sliced-up samples were placed in glass-stoppered weighing bottles and covered with alcohol. The alcohol was then driven off and the samples dried to constant weight. All data were calculated to the original wet weight of the potato used. The potatoes used in the experiment were smooth white potatoes usually purchased at the local market. The cultures of fungi used in the experiments were subcultures from Carpenter's cultures of *F. oxysporum* 3395 and 3315; *F. radicicola* 3113 and 3319, and *F. coeruleum* 3501.

EXPERIMENTATION

To determine the amount of variation in content of the different compounds in the four quarters of the potato, series of preliminary analyses were carried out. In these the potato was sampled in the usual way, except that the portions were sliced immediately and prepared for analysis. The results of these analyses are shown in Tables I to VI.

TABLE I.—*Reducing sugar and sucrose content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Reducing sugar.				Sucrose.			
	Quarter A.	Quarter B.	Quarter C.	Quarter D.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
43	0.10	0.11	0.09	0.11	0.04	0.02	0.04	0.02
44	.06	.06	.07	.06	.04	.04	.03	.03
46	.17	.14	.14	.19	.02	.03	.04	.04
49	.02	.02	.03	.02	.03	.03	.03	.04
87	0	0	0	0	.07	.07	.07	.06
88	0	0	0	0	.06	.05	.06	.05
105	0	0	0	0	.05	.08	.07	.06

TABLE II.—*Starch content of quarters of sound potatoes determined by the direct acid-hydrolysis method*

[Expressed as percentage of starch, wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
71	17.88	19.52	17.07	17.26
70	16.43	15.35	15.64	16.04
47	15.04	16.04	16.00	14.50
50	18.56	17.16	16.54	17.27

TABLE III.—*Pentosan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
19	0.51	0.43	0.46	0.48
28	.37	.35	.35	.37
47	.41	.40	.37	.40
207	.51	.48	.48	.51

[1] Wiley, H. W., ed. Op. cit.

TABLE IV.—*Galactan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
118	0.025	0.030	0.034	0.027
135	.028	.020	.027	.029
120	.034	.024	.030	.025

TABLE V.—*Crude fiber content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
102	0.50	0.59	0.48	0.47
210	.47	.47	.47	.44
211	.40	.41	.39	.36

TABLE VI.—*Dry matter in the quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
118	20.86	19.96	21.95	17.78
119	20.70	20.96	19.16	19.77
120	19.73	20.23	21.42	20.73
138	24.12	24.58	25.36	24.80
143	24.19	22.37	24.81	22.82

Tables I to VI show that there is considerable variation in the percentage of some of the compounds in different quarters of the same tuber, though usually the actual difference is not great. It is noticeable that two portions of the same tuber are more nearly alike in composition than samples from different potatoes. The method, therefore, which involves the comparison of the content of two quarters of the same potato is more accurate than one based on a comparison of the composition of two different potatoes. The experiments in which sound and rotted quarters were analyzed to determine the effect of the fungi upon the potato show that data from which definite conclusions may be drawn can be obtained by this method.

Inasmuch as the mycelium of the fungi was present in the rotted portions of the potatoes, it was of interest to determine what influence the compounds elaborated by these fungi would have on the apparent composition of the tuber. Quantities of mycelia of the two fungi *F. radicicola* and *F. oxysporum* were accordingly grown on potato extract. This medium was prepared by boiling sliced potatoes until they were soft, filtering the extract through cotton, and sterilizing it in suitable flasks.

The flasks of this medium were inoculated and the fungi allowed to grow for two or three weeks. The mat of mycelium was then removed, washed, dried, ground, and analyzed. The data obtained from these analyses, calculated as percentage of the dry weight, are given in Table VII.

TABLE VII.—*Amount of alcohol-insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid, pentosans, methyl pentosans, galactans, and crude fiber in mycelium of Fusarium oxysporum and Fusarium radicicola*

[Expressed as percentage of dry weight]

Species.	Alcohol - insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid (as dextrose).	Pentosans.	Methyl pentosans.	Galactans.	Crude fiber.
Fusarium oxysporum..	34. 58	2. 53	0. 73	0. 86	21. 8
	31. 90	2. 60	. 68	. 66	18. 4
Fusarium radicicola....	31. 63	1. 20	1. 50	. 72	20. 3
	31. 48	1. 20	1. 50	. 64	17. 6

It is apparent from Table VII that the fungi growing on the culture media prepared from potatoes produce pentosans, methyl pentosans, galactans, and a considerable quantity of substance which is insoluble in alcohol and reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid. That this last-mentioned substance can not result from the hydrolysis of the pentosans is evident from the relatively small pentosan content of the mycelium. The amount of substance which is considered as crude fiber in the table is also quite marked. It is evident, then, that both fungi build compounds which may be expected to raise the content of pentosans, galactans, and other substances in the tissue of the potato when the fungi and host are analyzed together. It must be remembered, however, that the percentages given in Table VII are related to dry weight of washed fungus mycelium and that the content of mycelium in 25 gm. of wet weight of the potato rotted with either of these fungi would be small.

The general appearance of the rotted portion of potato was typical for tubers rotted with these fungi at laboratory temperatures (from 20° to 25° C.) in a saturated atmosphere—that is, it was a wetrot (4, p. 187). The skin apparently was uninjured and could have been removed entire in most cases. The inner portion was soft and generally disorganized. Microscopic examination showed that the cells of the interior were apparently free from each other, as if the middle lamellæ had been dissolved. The starch grains did not appear to have been eroded in the time allowed for the experiment. The method of preparing the quarters of potato for analysis has been described.

The starch and sugar determinations were usually made on the same portion by extracting the pulp with alcohol, the extract being used for

the sugar and the solid residue for the starch determinations. The effect of three species of Fusarium, *F. oxysporum*, *F. radicicola*, and *F. coeruleum*, on the starch and sugar content of sound and rotted quarters of the same tubers was studied. The data obtained from the determination of the sugars are shown in Table VIII.

TABLE VIII.—*Reducing sugar and sucrose content of the sound and rotted quarters of potatoes*

[Expressed as percentage of the original wet weight]

Species of Fusarium and potato No.	Reducing sugar.		Sucrose.	
	Rotted quarter.	Sound quarter.	Rotted quarter.	Sound quarter.
Infected with *Fusarium oxysporum:*				
160	0.04	0.31	0.10	0.66
159	.04	.28	0	.67
158	0	.44	0	1.03
Infected with *Fusarium coeruleum:*				
149	.13	.40	.12	.39
150	.04	.47	.24	.50
151	.17	.37	0	.66
Infected with *Fusarium radicicola:*				
32	0	.03	.04	.24
26	0	.02	.04	.19
34	0	.03	.02	.09
41	0	.02	0	.42

In Table VIII it may be seen that all three species of Fusarium used the sugars. In most cases practically all the sugar had disappeared from the rotted portion, the cane sugar being utilized almost if not quite as completely as the reducing sugars. That the fungi could use disaccharids directly—that is, without breaking them down to their constituent monosaccahrids—seemed unlikely. It was therefore probable that the fungi secreted enzyms which were capable of hydrolyzing cane sugar, and possibly maltose also. To determine this point, tests were made for sucrase and maltase in extracts of the mycelium of *F. oxysporum* and *F. radicicola*. The fungi were grown for about three weeks or until a thick mat of mycelium was formed on potato extract. The felt was then separated from the liquid, ground up in a mortar and digested for 48 hours under toluol. The extract was filtered off and portions of it added to solutions of the sugars of known concentration. Controls of the boiled extract were also prepared. After the preparations had been allowed to stand overnight at laboratory temperature the amount of reducing sugar was determined. It was found that in the preparations of unboiled extract the sugars, both sucrose and maltose, were inverted almost quantitatively. The boiled extracts were practically without effect. It is evident then that the two fungi secrete both sucrase and maltase.

The starch determinations in the sound and rotted portions of the same tuber were made by two methods, as has been said. The data obtained by the direct acid hydrolysis method are given in Table IX, while the results of the determinations by the diastase method with subsequent acid hydrolysis are shown in Table X.

TABLE IX.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium, as found by direct acid hydrolysis*

[Expressed as percentage of starch of the original wet weight]

Potato No.	*Fusarium oxysporum.*		Potato No.	*Fusarium coeruleum.*		Potato No.	*Fusarium radicicola.*	
	Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.
165	14. 40	13. 53	149	19. 19	18. 69	34	16. 18	15. 79
168	14. 08	14. 67	150	18. 72	17. 48	26	15. 24	16. 60
180	16. 16	14. 62	151	22. 06	22. 22	32	15. 15	16. 01
						41	16. 83	16. 85

TABLE X.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium as determined by the diastase method with subsequent acid hydrolysis*

[Expressed as percentage of starch, wet weight]

Potato No.	*Fusarium oxysporum.*		Potato No.	*Fusarium radicicola.*	
	Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.
25	17. 77	16. 85	47	17. 50	16. 66
33	12. 50	11. 32	48	16. 66	15. 16
27	15. 32	14. 05			

The effect of the fungi upon the starch in the potatoes is in marked contrast to their action on the sugars. In Table IX, which gives the results of starch determinations by the direct acid-hydrolysis method, it may be seen that the starch content of the rotted portion appears to be higher in many cases than that of the corresponding sound quarter. In the determinations by the diastase method followed by acid hydrolysis the apparent starch content of the rotted portion is always higher, as shown in Table X. The fact that the fungi build up substances which are insoluble in alcohol and reduce Fehling's solution when hydrolyzed with dilute hydrochloric aicd, as shown in Table VIII, would account for any apparent increase in starch content in the rotted portion when the starch is determined by the direct acid-hydrolysis method. If the substances are also either soluble in hot water originally or made so by the diastase treatment, the apparent increase in starch content when the starch is determined by this method would be explained. In the diastase method the starch paste is liquefied by the action of the diastase,

then filtered, and the filtrate hydrolyzed with dilute hydrochloric acid. Some of the mycelium of these fungi was extracted with alcohol, and then dried and extracted with hot water. The extract was then filtered off, treated with hydrochloric acid exactly as in the acid hydrolysis of starch, neutralized and tested for reducing substances. A considerable quantity was found. The filtrate did not give a qualitative test for pentosans. The apparent increase in starch content in the rotted portions of the potatoes, then, is due to compounds laid down by the fungi. From the fact that only a small amount of mycelium of these fungi could be present in the rotted potato it would seem probable that if the starch were attacked to any extent the apparent starch content as obtained by acid hydrolysis would be lowered in all cases. To obtain further information on this point experiments were carried out to ascertain whether these fungi secreted diastase and if so, whether this enzym could break down the starch grains of the potato.

Extracts of the undried, ground mycelium of the two fungi, *F. oxysporum* and *F. radicicola*, were made with 50 per cent glycerin. These extracts were filtered after 24 hours through absorbent cotton and portions added to a 2 per cent solution of "soluble starch." Suitable controls were prepared and all preparations allowed to stand in an incubator under toluol at 30° C. for 48 hours. At the end of this time the starch was practically all broken down by the extracts of both fungi. Similar experiments were carried out with starch paste made from potato starch with positive results. The fungi then secrete diastatic enzyms. The experiments, however, did not prove that the diastases were able to attack the starch grains before they were broken down. Brown and Morris (2) have shown that malt diastase can not act on ungelatinized potato starch, though the starch grains of barley are readily eroded by it. Whether the enzyms in the extracts of the mycelium could erode the starch grains of the potato at room temperature was determined by placing some well-washed potato starch in extracts and allowing the preparations to stand under toluol. They were shaken up and examined from time to time, but no sign of erosion of the starch grains was evident even at the end of a week. The extracts used were tested on starch paste or "soluble starch" with positive results. Smith and Swingle (9) mention that the starch in the potatoes rotted with *F. oxysporum* was apparently not eroded. It is, of course, possible that the potato starch grains are very slowly attacked by the diastases of these fungi or that some inhibitor is present which prevents the action of the enzym on the starch in this condition at the temperature at which these studies were made. These points should be investigated. At present, however, the conclusion seems warranted in view of the evidence that the starch of the potato is not appreciably affected by the fungi.

From the fact that these fungi penetrate the cell walls or parts of the cell walls of the potato in living parasitically upon their host, their effect

on the constituents of the cell wall was considered of especial interest. The substances studied in this investigation which may be considered to be, in part at least, components of the cell walls are the pentosans, crude fiber, and galactans (5). Inasmuch as the fungi apparently do not affect the skin in rotting the potato, it was considered of interest to find out the relative distribution of the pentosans and crude fiber in the skin and inner portion of the potato. For these analyses the potatoes were peeled as thinly as convenient and determinations made on the weighed peeling and inner portion separately. The results of the pentosan determinations are given in Table XI.

TABLE XI.—*Pentosan content of the peeling and inner portion of potatoes*

[Expressed as pereentage of pentosans, wet weight]

Potato No.	Skin.	Inner portion.	Potato No.	Skin.	Inner portion.
116	0.62	0.28	140	1.02	0.59
133	.88	.39	163	.72	.36
134	.80	.47	164	1.07	.50

When the pentosan content is calculated as wet weight, it is about half as great in the inner portion of the tuber as in the skin. There is, nevertheless, a considerable amount of the furfurol-yielding compounds in the fleshy part of the potato. Inasmuch as the fungus has practically no effect on the skin, it is to be considered that practically all changes in the pentosan content that take place during rotting are in the inner portion of the tuber. The results obtained from the pentosan determinations on the sound and the rotted portions of the potato tubers are shown in Table XII.

TABLE XII.—*Pentosan and methyl-pentosan content of sound and rotted quarters of potatoes*

[Expressed as percentage of pentosans, wet weight]

Potato No.	Sound quarter.			Rotted quarter.		
	Total pento-sans.	Pen-tosans.	Methyl pen-tosans.	Total pento-sans.	Pen-tosans.	Methyl pen-tosans.
Infected with *F. oxysporum:*						
29	0.53	0.47	0.06	0.50	0.35	0.15
30	.53	.41	.12	.46	.35	.11
35	.45	.36	.09	.44	.35	.09
40	.52	.42	.10	.37	.26	.11
Infected with *F. radicicola:*						
171	.28	.23	.05	.25	.20	.05
174	.37	.32	.05	.29	.24	.05
176	.25	.19	.06	.26	.21	.05

Table XII shows that the total pentosan content, which includes all furfurol-yielding matter, and the pentosan content, which is the total pentosan content after the methyl pentosans have been extracted, are higher in all but one instance in the sound portions of the tuber. There is slightly more variation in methyl pentosan content; it is the same or greater in the rotted as in the sound portion in all but two cases. The fungi evidently use the pentosans, but do not affect the methyl pentosans to any extent. It is to be remembered that these fungi build up both pentosans and methyl pentosans when growing on potato extract. The content of these substances, then, in the rotted portions given in Table XII is undoubtedly the difference between the amount of pentosans broken down by the fungi in the interior of the potato and the amount built up by the fungi. The destructive processes evidently proceed more rapidly than the constructive, and some of the pentosans of the potato are used either in respiration or in the building up of other compounds.

From the effect of the fungi on pentosans it was considered probable that enzyms which could hydrolyze these compounds were present in the mycelium. Experiments were undertaken to determine this point.

The experiments were carried out as described in a previous paper (8), except that the fungi were grown on potato extract instead of a synthetic medium with gum arabic as a source of carbon. Xylan from rye straw was used as a substrate. The results of these experiments are given in Table XIII.

TABLE XIII.—*Effect of boiled and unboiled extract of mycelium upon xylan from rye straw, as shown by alcohol-soluble furfurol-yielding material and substance reducing Fehling's solution. (0.2 gm. of xylan in each preparation was maintained at 30° C. for one week.)*

Species of Fusarium.	Quantity of cuprous oxid derived from material reducing Fehling's solution.		Quantity of alcohol-soluble furfurol-yielding substances as pentosans.	
	Unboiled.	Boiled.	Unboiled.	Boiled.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
Fusarium radicicola	45.2	15.4	13.1	5.7
	44.8	14.8	13.1	5.7
Fusarium oxysporum	14.1	6.5	18.6	6.8
	16.4	6.5	14.6	6.8

It is evident from Table XIII that the extracts of the fungi are able to break down xylan prepared from rye straw to an alcohol-soluble compound which reduces Fehling's solution and which forms furfurol when boiled with hydrochloric acid. The fungi then secrete an enzym or enzyms which can break down xylan probably to xylose.

The crude fiber of the potato is undoubtedly a mixture of compounds, among which are some of the cell wall constituents, including whatever

cellulose may be present. The distribution of the crude fiber throughout the tuber is not as uniform as that of the pentosans, as is shown by a comparison of Tables XI and XIV.

TABLE XIV.—*Crude fiber content of the skin and inner part of the potato tuber*

[Expressed as percentage on both a wet weight and dry weight basis]

Potato No.	Percentage of crude fiber, wet weight.		Percentage of crude fiber, dry weight.	
	Skin.	Inner portion.	Skin.	Inner portion.
210	1.54	0.25	11.11	1.16
211	1.33	.25	6.61	1.06
212	1.20	.36	7.89	1.82

From Table XIV it may be seen that the crude-fiber content of the peeling is 3½ to 6 times greater than that of the inner portion calculated on a wet weight basis and from 4 to 10 times greater on the basis of dry weight. The inner portion of the potato contains usually a lower percentage of crude fiber than of pentosans.

The determinations of crude fiber on the sound and rotted portions of the potato tubers are given in Table XV.

TABLE XV.—*Crude-fiber content in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola*.			Rotted with *Fusarium oxysporum*.		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
37	0.56	0.54	177	0.71	0.58
39	.57	.56	178	.73	.62
115	.40	.37	179	.69	.62

The crude-fiber content is always higher in the rotted quarter of the tuber than in the corresponding sound portion, though the difference is not great. As has been mentioned earlier in this paper, the fungus builds up a considerable quantity of substance which is not dissolved in either the acid or alkali used in the crude-fiber determination; to this is due the rise in the crude-fiber content of the potato during rotting. It is possible, of course, that the fungi may break down the crude fiber of the host plant and build up some similar substance with greater rapidity. From the evidence brought out in these experiments, then, it is impossible to draw definite conclusions.

The substances in the potato which give mucic acid when boiled with proper concentration of nitric acid are considered in this study as galactans. They are present in small quantities in the potato, and the com-

bination in which they occur in the tuber was not investigated. Galactose might occur in combination with raffinose, in a glucoside or combined in the cell walls. It probably occurs in plants most commonly in the last-mentioned combination. The effect of the fungi upon the galactan content of the potato is shown in Table XVI.

TABLE XVI.—*Galactan content of sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola.*			Rotted with *Fusarium oxysporum.*		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
27	0.039	0.062	166	0.069	0.071
31	.033	.060	167	.068	.076
42	.029	.030	172	.081	.083

It is evident from the table that the fungi lower the galactan content of the potato. The fungi produce galactans when growing upon potato extract and the data in Table XVI show that the breaking down process proceeded faster than the building up.

The amount of dry matter of the sound and rotted quarters determined as mentioned earlier in this paper is shown in Table XVII.

TABLE XVII.—*Amount of dry matter in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola.*			Rotted with *Fusarium oxysporum.*		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
27	20.83	21.19	166	17.73	18.91
31	19.88	22.59	167	18.93	20.45
42	20.98	22.13	172	18.17	19.36

As was to be expected, the rotting of the potato by the fungi lowered the percentage of dry weight as calculated to the original weight of the portion of the potato used in the experiment. This is probably due to an increased respiration—that is, a respiration of the quarter of the potato plus the respiration of the fungus which in a given time is greater than a portion of the same potato alone.

DISCUSSION

From the foregoing pages it is evident that the tuber-rot fungi used in this study considerably alter the composition of the potato. That they should be able to utilize the sugars of the potato was to be expected. Most fungi use glucose readily as a source of carbon. Behrens (1) has shown that *Sclerotinia fructigenia* lowers the sugar content of apples in

rotting them. The brownrot fungus of peaches reduces the sugar content of that fruit. The presence of the enzyms sucrase and maltase in fungi has frequently been recorded.

The starch content of the potato makes up the greater part of its dry weight and may be regarded as stored food material. That the fungi which so efficiently utilize the monosaccharids and disaccharids of the potato tuber are unable, apparently, to affect this polysaccahrid is of considerable interest. The fungi grow for the most part in the cell walls and thus are not closely in contact with the starch grains. This might retard the action because of the low rate of diffusion of the diastase but could hardly inhibit it entirely. The fact that the diastases of these fungi had no apparent effect on unbroken starch grains *in vitro* during the time of the experiment, while potato starch when gelatinized was readily hydrolyzed by these enzyms, indicates that the rate of action under what are usually favorable conditions for such reaction is to say the least very low. The experiments seem to show that enzymic studies are of doubtful value in determining the effect of the parasite on the host plant unless corroborated in a study of the physiological relations existing between the two organisms. The effect of the fungi on the pentosan and galactan content of the potato shows that they can break down at least some of the constituents of the cell wall. Now, when a parasitic fungus such as those used in this study enters a cell of its host plant, it must either force its way in mechanically by exerting sufficient pressure to rupture the cell wall or a portion of the cell wall must be dissolved. Likewise, in growing between the cells of the host plant where no appreciable intercellular spaces exist, the cells must be forced apart mechanically or some parts of the cell walls dissolved. It is evident from their effect on the pentosans that these fungi are able to dissolve at least some portions of the cell wall. That they secrete enzyms which can hydrolyze xylan is more evidence on this point. The crude-fiber content of the potato was increased in rotting owing to the formation in the fungi of some substances which were not broken down by the acid or alkali treatment in the crude-fiber determinations. Therefore it was impossible to obtain evidence as to the effect of the fungi upon the crude fiber. As shown in the tables the crude-fiber content of the inner portion of the potato is not high. It is noticeable that throughout this study the different species of Fusarium had practically the same effect on the potato.

CONCLUSION

In conclusion, it has been shown in this study that the fungi in the potato reduced the content of sugar, both sucrose and reducing sugar, pentosans, galactans, and dry matter. The starch and methyl pentosans are apparently not affected appreciably, and the crude-fiber content was not reduced. It was shown that these two species of fungi secrete sucrase, maltase, xylanase, and diastase; the last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch.

LITERATURE CITED

(1) BEHRENS, Johannes.
1898. Beiträge zur Kenntnis der Obstfäulnis. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, No. 17/18, p. 700–706.

(2) BROWN, H. T., and MORRIS, G. H.
1890. Researches on the germination of some of the Gramineae. *In* Jour. Chem. Soc. [London], v. 57, Trans., p. 458–528, 2 pl.

(3) BRYAN, A. H., GIVEN, A., and STRAUGHN, M. N.
1911. Extraction of grains and cattle foods for the determination of sugars: a comparison of the alcohol and the sodium carbonate digestions. U. S. Dept. Agr. Bur. Chem. Circ. 71, 14 p.

(4) CARPENTER, C. W.
1915. Some potato tuber-rots caused by species of Fusarium. *In* Jour. Agr. Research, v. 5, no. 5, p. 183–210, pl. A–B (col.), 14–19.

(5) CZAPEK, Friedrich.
1905. Biochemie der Pflanzen. 2 Bd. Jena.

(6) ELLETT, W. B., and TOLLENS, B.
1905. Ueber die Bestimmung der Methyl-Pentosans neben den Pentosanen. *In* Ber. Deut. Chem. Ges., Bd. 38, No. 2, p. 492–499.

(7) HAWKINS, L. A.
1915. Some effects of the brown-rot fungus upon the composition of the peach. *In* Amer. Jour. Bot., v. 2, no. 2, p. 71–81. Literature cited, p. 80–81.

(8) ———
1915. The utilization of certain pentoses and compounds of pentoses by Glomerella cingulata. *In* Amer. Jour. Bot., v. 2, no. 8, p. 375–388.

(9) SMITH, Erwin F., and SWINGLE, D. B.
1904. The dry rot of potatoes due to Fusarium oyxsporum. U. S. Dept. Agr. Bur. Plant Indus. Bul. 55, 64 p., 1 fig., 8 pl.

(10) WOLLENWEBER, H. W.
1913. Studies on the Fusarium problem. *In* Phytopathology, v. 3, no. 1, p. 24–50, 1 fig., pl. 5. Literature, p. 46–48.

HYPERASPIS BINOTATA, A PREDATORY ENEMY OF THE TERRAPIN SCALE

By F. L. Simanton,
Entomological Assistant, Deciduous Fruit Insect Investigations, Bureau of Entomology

INTRODUCTION

One of the most effective enemies of lecanium scales is the coccinellid beetle *Hyperaspis binotata* Say. Its economic importance was impressed on the writer during the seasons of 1912 and 1913, when he was studying the life history and control of the terrapin scale (*Eulecanium nigrofasciatum* Pergande). Throughout the spring and early summer the larvæ, conspicuous by their flocculent covering, could be found in large numbers feeding upon the immature scales and overturning the adult scales. The adult beetles do not feed upon the mature scales, but they destroy the young and also attack aphides, or plant lice, and other soft-bodied insects. In view of the economic importance of this beetle a study of its life history was undertaken at the suggestion of Dr. A. L. Quaintance, in charge of Deciduous Fruit Insect Investigations, Bureau of Entomology. The work was begun in the summer of 1912 and completed in 1913.

HISTORICAL SUMMARY

Very little has been written about *Hyperaspis binotata*. Say (1, p. 303),[1] in 1826, described the male under the present name, and the female as *Coccinella normata*. G. R. Crotch (2, p. 380) considered the form with the subapical red spot as a variety of *H. signata* Olivier, and gave as synonyms *H. binotata* Say, *H. normata* Say, and *H. leucopsis* Melsheimer.

T. L. Casey (3, p. 124), in 1899, considered *H. binotata* Say as a distinct species and gave the following synonymy: *H. signata* Le Conte, *H. normata* Say, *H. affinis* Randall, and *H. leucopsis* Melsheimer.

J. G. Sanders (4, p. 3), in 1905, mentions *H. binotata* as a valuable predatory enemy of *Pulvinaria* spp. J. B. Smith (5, p. 606; 6, p. 570), in the same year, reported the same species as reducing an infestation of *Pulvinaria* spp. at Montclair, N. J., from 500 to 1,000 scales to a leaf to about one dozen scales to a leaf.

S. A. Forbes (7), in his annual report for 1908, mentions the species as one of the principal enemies of *Pulvinaria* spp. in Illinois. In 1910, W. S. Blatchley (8, p. 523), gives a key to the species of Hyperaspis found in Indiana and remarks that *H. binotata* Say is "a variety of *H. signata* Oliv., having the subapical spot lacking, color and structure otherwise exactly as in that species." W. E. Britton (9, 8), in 1914, treats this species,

[1] Reference is made by number to "Literature cited," p. 203.

Journal of Agricultural Research, Vol. VI, No. 5
Dept. of Agriculture, Washington, D. C. May 1, 1916
do K—30

mentioning it as a great destroyer of the cottony maple scale (*Pulvinaria vitis* Linnaeus) and stating that it feeds upon both the woolly maple-leaf scale (*Phenacoccus acericola* King) and the tulip scale (*Eulecanium tulipiferæ* Cook).

These references bring the history of the species down to the date of the present paper, which deals with the life history and habits of the species when feeding upon the terrapin scale.

DISTRIBUTION

H. binotata occurs in most of the territory east of the Mississippi River and extends west of this river in some States to the semi-arid region. It is most abundant in the Atlantic States from Connecticut to Maryland, but is common from New Jersey to Illinois. All localities known to the writer are indicated upon the map (fig. 1).

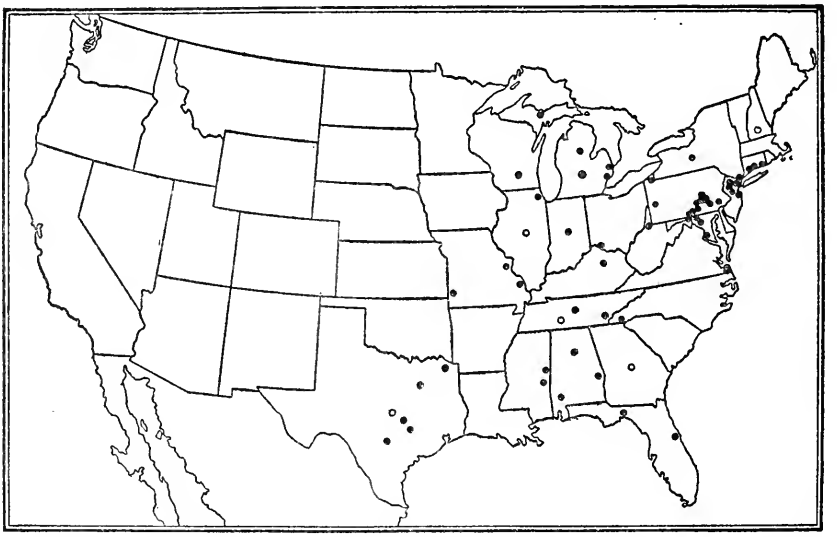


FIG. 1.—Map showing the distribution in the United States of *Hyperaspis binotata*: ●=definite record; ○=doubtful record.

HOSTS

H. binotata feeds upon honeydew, aphides, aphis eggs, and mealy bugs and other soft-bodied scales. The larvæ, so far as observed, feed upon scale larvæ and young scales. They seem to have preyed originally upon species of Pulvinaria, to the egg masses of which the larvæ have a superficial resemblance. The species thrives upon the terrapin scale and seems to be rather more abundant where it preys exclusively upon this scale.

DESCRIPTION OF LIFE STAGES

IMAGINAL STAGE

The adult (Pl. XXIV, fig. 1, 2) is a small hemispherical beetle which passes the winter in rubbish or under bark. It was described by Say (1) in 1826 from the male as follows:

"Black, lateral margin of the thorax and head yellow; each elytron with a rufous spot; body rounded-oval, convex, punctured, black, polished; head pale yellow, labrum and transverse line on the vertex piceous; thorax with a yellow margin extending for a short distance on the anterior margin; anterior margin with an obsolete yellowish line interrupted in the middle; elytron each with a rufous, orbicular, central spot."

EGG STAGE

The egg (Pl. XXIV, fig. 3), which was first obtained by the writer in 1913, is oblong-elliptical and somewhat depressed; 10 specimens measured from 0.6 to 0.775 mm. in length (average, 0.704 mm.) and from 0.218 to 0.4 mm. in width (average 0.312 mm.). In color it is light salmon, changing ultimately to ash-gray; the shell is membranous, becoming indented with age. Hatching takes place through a longitudinal slit on the upper surface.

LARVAL STAGE [1]

The first instar has characteristic markings, and represents a rather primitive type of coccinellid larva. The other instars are similar to the first, but they are covered by a white fleece of wax filaments which masks their characters.

FIRST INSTAR (Pl. XXIV, fig. 4).—Length 1.22 mm. (1.125 to 1.275 mm.), width 0.478 mm. (0.450 to 0.575 mm.); body grayish white, semiopaque, cylindrical, and tapering caudad. Head black, with a white trident spot over the epicranial and frontal sutures; three pairs of ocelli present; length 0.125 mm., width 0.225 mm. Thorax sparsely pilose, the segments each with a pair of black dots; prothorax with two black clouded areas surrounding, but mainly cephalad of the dots. Abdominal segments each with a row of eight hairs and a pair of long lateral setæ; ninth segment black above; tenth segment, the so-called anal lobe, retractile.

SECOND INSTAR (Pl. XXIV, fig. 3, *a*).—Length 2.5 mm. (1.3 to 2.75 mm.), width 1.08 mm.; body yellowish white, pubescent and covered with a white fleece. Head black with the trident spot mildly obscured; length 0.175 mm., width 0.325 mm. Thorax white, immaculate; legs gray, marked with black. Abdomen devoid of conspicuous lateral setæ.

THIRD INSTAR.—Length 2 to 3.38 mm., mostly 2.5 mm.; width 0.9 to 1.75 mm., mostly 1.125 mm. Head black, pigmentation on the posterior part of labium confluent; length 0.275 to 0.3 mm., width 0.45 to 0.5 mm., mostly 0.475 mm. Abdomen with eight pairs of conspicuous blood pores. Otherwise as in the second instar.

FOURTH INSTAR (Pl. XXV, fig. 1, 2).—Length 2.5 to 6.25 mm., mostly 5.5 mm.; width 1.125 to 2.5 mm., mostly 2.25 mm. Body subglobose, yellowish gray. Head glabrous, white, flecked with black, pigmentation on the posterior part of labium not confluent on the median line; length 0.3 to 0.375 mm., mostly 0.35 mm.; width 0.575 to 0.65 mm., mostly 0.6 mm. Otherwise as in the third instar.

PUPAL STAGE

Pupa (Pl. XXV, fig. 3, 4) inclosed within the larval skin; length 2.03 to 4.19 mm., mostly 3.9 mm.; width 1.77 to 1.86 mm.; color uniform chestnut-brown; ovate, with a depressed segmented area on the dorsum; dorsal surface hispid; ventral surface mildly pilose.

[1] A detailed morphological study of this larva by Dr. Adam Böving is in course of preparation.

HABITS AND SEASONAL HISTORY

THE BEETLES

The beetles emerge from hibernation at Mont Alto, Pa., about the middle of April and commence mating about the 20th of that month. When the species is feeding upon the terrapin scale, the beetles hibernate for the most part at the bases of scale-infested peach (*Amygdalus persica*) trees. After emerging from hibernation they soon depart in search of food and do not return to the peach until the adult scale, which the beetle is unable to destroy, begins to deposit honeydew—about the middle of May. For the rest of the season the species remains upon the peach, feeding upon the scale and its honeydew. The overwintering beetles are nearly all dead by the middle of July, while the new brood of beetles escapes from pupæ for the most part during the first half of that month.

There is some indication of a second brood, but there is not enough evidence at hand to establish it.

THE EGGS

A very typical group of four eggs just as they were deposited is shown in Plate XXIV, figure 3. It will be noticed that the eggs are not clustered, but are placed more or less at random in the irregularities of the bark adjacent to the host. The terrapin scale upon which the species was feeding is found only upon young wood, the growth rings of which supply a convenient shelter for the eggs of the beetle. It is not unusual, however, to find eggs in crevices at the base of fruit spurs or even upon smooth bark. It is worthy of note in this connection that the eggs are not placed under the scales. It was found that the membranous shell became dry and shriveled in from three to six days, and that the egg changed to an ash-gray near the end of the incubation period.

The first eggs of the season were laid upon the twigs of scale-infested peach trees at Mont Alto, Pa., on May 3, 1913, but were immediately consumed by the beetles, as were all later eggs, until the food supply became abundant. It was not until May 26 that eggs were permitted to hatch. Oviposition reached its maximum about June 5, and continued in a small way until September 1. Owing to the tendency of the beetles to devour their eggs, it was not possible to determine definitely the beginning of oviposition or the total number of eggs; 36 was the largest number obtained from a single female, but there were indications that several times that number had been deposited. Incubation lasts from six to eight days; the average for 18 eggs deposited between June 27 and 30, 1913, was seven days.

THE LARVÆ

The larvæ at the time they escape from the egg have the pigment lacking from the head, legs, and ninth abdominal segment. They begin searching at once for the terrapin scales; and when one is found, a larva enters the brood chamber through the anal cleft, where it remains during the first and second instars. The first noticeable appearance of the coccinellid larvæ in the orchard, which occurs about June 18, coincides with the beginning of reproduction of the terrapin scale. Once within the brood chamber of a scale the coccinellid larva (Pl. XXIV, fig. 4) preys upon the new-born young of that particular scale until the end of the second instar, by which time the rapidly growing coccinellid displaces the scale.

The second molt is made in the open, mostly at the base of a fruit spur. In the third and fourth instars many mature scales are destroyed, being displaced (Pl. XXIV, fig. 5) by the coccinellid larvæ as these thrust their heads into the brood chambers to secure the young scales. When all the old scales have been destroyed, the ladybird larvæ, which now have a superficial resemblance to mealy bugs, migrate to the leaves and there continue to feed upon such of the scale larvæ as were able to reach the leaves. It is estimated that a single coccinellid larva will destroy 90 mature scales and 3,000 larvæ.

The length of the larval instars, together with the number of specimens used in their determination, is shown in Table I.

TABLE I.—*Length of the larval instars of Hyperaspis binotata*

Instar.	Number of specimens.	Length of instar.		
		Average.	Minimum.	Maximum.
		Days.	*Days.*	*Days.*
First	17	2. 98	2	4
Second	11	2. 18	1	3
Third	7	2. 71	2	4
Fourth	5	12. 00	12	12

The dates at which the respective instars occur in the field are given in Table II. The first and second dates show the time of greatest abundance; the first and last dates show the total time of occurrence for each instar.

TABLE II.—*Sequence of the seasonal appearance of the larval instars of Hyperaspis binotata in the field*

Instar.	Date present in field.
First	June 17 to 20 to Sept. 15.
Second	June 20 to 22 to Sept. 20.
Third	June 22 to 25 to Sept. 25.
Fourth	June 25 to July 7 to Sept. 30.

The author has depended upon head measurements in distinguishing the instars; a key for this purpose (Table III) has proved satisfactory. As will be seen from the table, it is only necessary to consider the width of the head.

TABLE III.—*Key for determining the larval instars of Hyperaspis binotata according to width of head*

Instar	Width of head.
	Mm.
First	0.225
Second	.325
Third	.475
Fourth	.600

THE PUPA

The pupal period lasts for from 10 to 13 days, averaging 12 days. Pupæ appear in the field early in July and are most abundant from the 7th to the 20th of the month. They are found, surrounded by the last larval skin, attached to leaves or concealed in clusters under bark. An occasional one may be found as late as October.

NATURAL ENEMIES

There seem to be very few enemies of this ladybird. No parasites were obtained, and no birds were observed to feed upon it. Aphis lions were found preying upon the eggs, and a common plant bug, *Brochymena* sp., was taken upon two occasions with this coccinellid impaled upon its beak.

SUMMARY

Hyperaspis binotata Say is found in the eastern United States and westward to the semiarid region. It feeds upon aphides and soft-bodied scales and is very effective in controlling the cottony maple scale and the terrapin scale. The eggs are salmon-colored and are deposited singly on twigs adjacent to the hosts. The life cycle requires 39 days and is as follows: Incubation, 7 days; first instar, 3 days; second instar, 2 days; third instar, 3 days; fourth instar, 12 days; pupa, 12 days.

LITERATURE CITED

(1) SAY, THOMAS.
1826. Descriptions of new species of coleopterous insects inhabiting the United States. *In* Jour. Acad. Nat. Sci., v. 5, p. 293–304.

(2) CROTCH, G. R.
1873. Revision of the Coccinellidae of the United States. *In* Trans. Amer. Ent. Soc., v. 4, p. 363–382.

(3) CASEY, T. L.
1899. A revision of the American Coccinellidae. *In* Jour. N. Y. Ent. Soc., v. 7, p. 71–163.

(4) SANDERS, J. G.
1905. The cottony maple scale. U. S. Dept. Agr. Bur. Ent. Circ. 64, 6 p., 4 fig.

(5) SMITH, J. B.
1906. The cottony maple scale. Enemies of the insect. *In* N. J. Agr. Exp. Sta. Ann. Rpt. [1904]–5, p. 605–607, illus.

(6) ———
1907. The signate lady-bird beetle. *In* N. J. Agr. Exp. Sta. 27th Ann. Rpt. [1905]–6, p. 570–572, illus.

(7) FORBES, S. A.
1908. Cottony maple scale in Illinois. Insect enemies. *In* 24th Ann. Rpt. State Ent. Ill., p. 114–115, illus.

(8) BLATCHLEY, W. S.
1910. On the Coleoptera known to occur in Indiana. 1,386 p., illus. Indianapolis. (Ind. Dept. Geol. and Nat. Resources Bul. 1.)

(9) BRITTON, W. E.
1914. Some common lady beetles of Connecticut. Conn. Agr. Expt. Sta. Bul. 181, 24 p. (p. 8), 24 fig.

PLATE XXIV

Hyperaspis binotata:

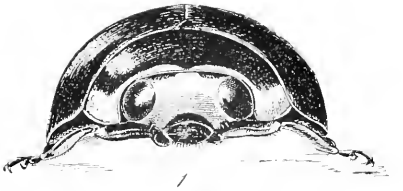
Fig. 1.—Male, showing the characteristic markings. Much enlarged.

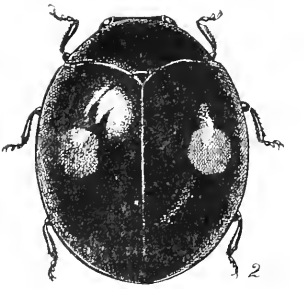
Fig. 2.—Female, showing the dorsal view. Much enlarged.

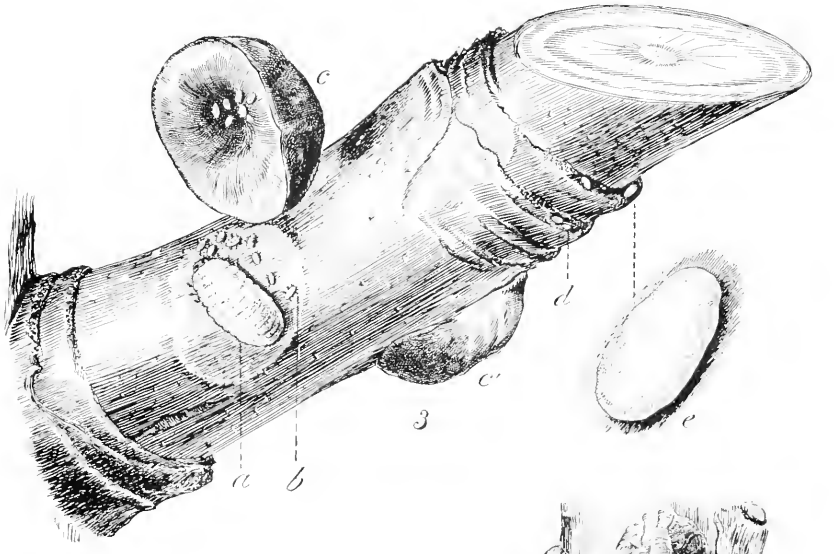
Fig. 3.—Eggs and a second-instar larva. *a*, Second-instar larva as disclosed by displacing the host; *b*, larvæ of the terrapin scale, *Eulecanium nigrofasciatum; c*, a displaced scale; *d*, eggs "in situ"; *e*, egg somewhat enlarged.

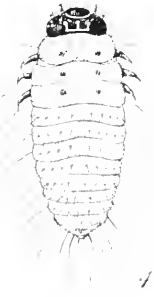
Fig. 4.—First-instar larva.

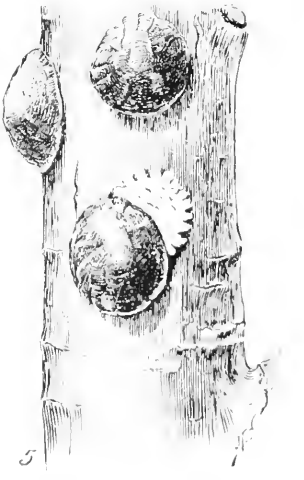
Fig. 5.—Method of attacking the mature scales during the third and fourth instars.

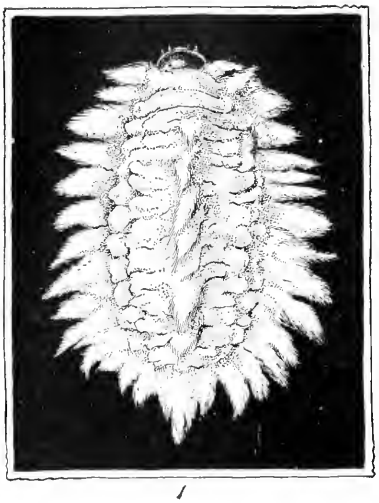

1


2

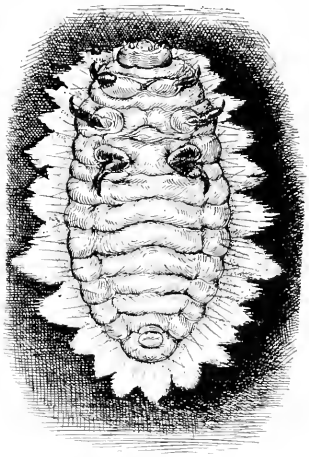

c
d
e
3
a
b


4


5

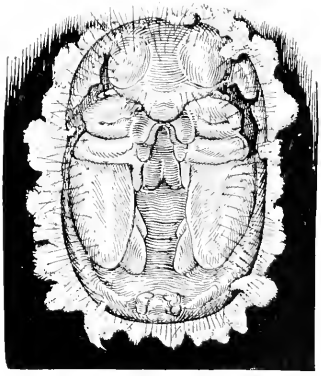


1



2

3



4

PLATE XXV

Hyperaspis binotata:

Fig. 1.—Mature larva as it appears when attacking the leaf-attached larvæ of the terrapin scale, *Eulecanium nigrofasciatum.*

Fig. 2.—Ventral view of mature larva.

Fig. 3.—Dorsal view of pupa, showing the last larval molt skin and the depressed segmented area.

Fig. 4.—Ventral view of pupa.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, $3.00 PER YEAR

Vol. VI MAY 8, 1916 No. 6

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., MAY 8, 1916 No. 6

TESTS OF THREE LARGE-SIZED REINFORCED-CONCRETE SLABS UNDER CONCENTRATED LOADING

By A. T. GOLDBECK, *Engineer of Tests*, and E. B. SMITH, *Associate Mechanical Engineer, Office of Public Roads and Rural Engineering*

INTRODUCTION

Numerous instances occur in reinforced-concrete design in which the use of slabs supported at two ends only is required, and in many such cases the critical loading is concentrated at one or more points. Such a condition may exist on slab-bridge floors, box culverts, on floors of buildings where heavy machinery is housed, and in other constructions where loads are concentrated.

If a slab, supported at two ends and carrying a single concentrated load, is imagined to be divided into narrow strips extending from support to support, it would seem reasonable to assume that the strip immediately under the load carries a very large part of it and that the adjacent strips receive a smaller amount, depending upon their distances from the load. The most remote strips, those at the edges of the slab, would then probably receive very little load. The question which concerns the designer of such a slab is that of the relative magnitude of the stresses at different distances from the load.

Up to a few years ago the technical literature on this subject was practically nonexistent, and the result was that engineers relied largely on their judgment when called upon to design slabs subjected to concentrated loads. Very naturally, large variations in load-distribution assumptions were made, and as a consequence there were great differences in the design even when the span and load to be carried were practically identical.

The necessity for definite knowledge on this subject was very forcibly brought to the attention of the engineers of the Office of Public Roads and Rural Engineering a few years ago, and a set of tests was made by one of the authors on slabs of 3-foot and 6-foot span length.[1] These tests gave some useful and rather surprising results that have since been

[1] Goldbeck, A. T. Tests of reinforced-concrete slabs under concentrated loading. *In* Amer. Soc. Testing Materials, Proc. 16th Ann. Meeting 1913, v. 13, p. 858–873, 10 fig. 1913. Discussion, p. 874–883, 4 fig.

verified; and in order to carry the investigation farther, with slabs of longer span than those previously investigated, the present series of tests was undertaken at the Arlington Experimental Farm of the United States Department of Agriculture.

OBJECT OF INVESTIGATIONS

The theory applied to the design of narrow rectangular reinforced-concrete beams involves the assumption that the stress is constant throughout the width of the beam. In a wide slab the stress distribution varies from a maximum at the point of application of the load to a minimum at the extreme edges. Obviously then, if the rectangular-beam theory were applied to the design of slabs under concentrated loads, the width b used in the design formulas can not be taken as the entire width of the slab. The rectangular-beam theory, however, could be utilized in wide-slab design if it were known what width b should be substituted in the design formulas, and it is the object of this paper to explain tests for determining this width and to demonstrate the application of the theory of narrow rectangular beams to the design of wide slabs supported at two ends and subjected to concentrated loads.

EFFECTIVE WIDTH

The width of the slab that should be used in the rectangular-beam formulas when applied to slab design will be termed the "effective width" of the slab. It is that width over which, if the stress were constant and equal to the maximum stress under actual conditions, the resisting moment would equal the resisting moment of a slab of the same depth and full width, but having varying stress distribution. If the straight-line theory of stress distribution from neutral axis to upper fibers is assumed to be applicable to slabs, the resisting moment of a given slab is dependent on the total stress in the concrete or steel at the dangerous section. The total stress in the concrete, however, is governed by the stresses in the top fibers, and these stresses are proportional to the unit deformations. If, then, there are two slabs of equal depth, one having uniform distribution of deformations and the other a varying distribution, but with their maximum deformations identical, they will likewise have equal resisting moments if the summations of the deformations over their respective widths are identical.

In figure 1, which represents a slab in position on two supports with a concentrated load P, is illustrated the method of obtaining "effective width." Strain-gauge readings are taken of the fiber deformations perpendicular to the supports, as indicated at *eg*. These concrete deformation values are plotted to scale, as, for instance, at *fh*, giving the deformation curve JHF, inclosing the area $AJHFE$. This curve shows the variation of stress from the center to each of the two free edges of the slab, and the area under the curve is a function of the total concrete-resisting

moment of the slab. The area *BDGI*, obtained by dividing the area *AJHFE* by its maximum ordinate *CH*, has the same total concrete-resisting moment with the stress uniformly distributed as the whole slab, and its width *BD* is that which may be effective in furnishing sufficient resistance under these conditions to carry the load. The width *BD*, obtained in this manner, is the "effective width."

DESCRIPTION OF APPARATUS

LOAD-APPLYING APPARATUS.—The slabs tested were 32 feet wide, with a span length of 16 feet, and in order to accommodate such extraordinarily large test specimens it was necessary to build special apparatus. Two supports 32 feet long were constructed of reinforced concrete, and embedded in each of them at the center were two loop-welded eyes carrying four 24-inch 80-pound I beams 6 feet above the level of the supports

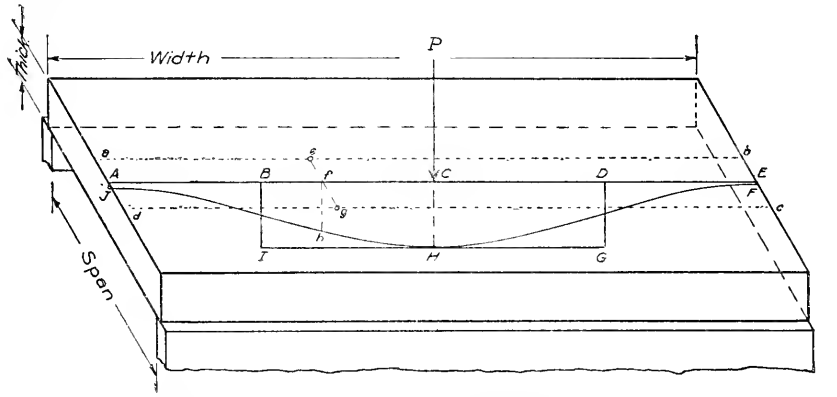


FIG. 1.—Diagram illustrating the method of obtaining "effective width" in reinforced-concrete slab tests.

(Pl. XXVI). Loads were applied by means of a hand-operated hydraulic jack mounted between the slab and the overhead I beams.

For weighing the loads a specially calibrated chrome-nickel beam (Pl. XXVI) was mounted between the jack and the load-applying I beams, and its deflection at the center was a measure of the load applied. This chrome-nickel beam was 7 inches wide, 5 inches deep, and 27 inches in span, and its deflection was measured with an Ames dial reading to 0.0001 inch. The dial was fastened to the beam and its plunger rested on a ½-inch square steel rod mounted on the side of the beam at the neutral axis. It was found that by fastening an electric buzzer on this rod more consistent readings could be obtained with the dial. The entire load-applying device was calibrated in a 200,000-pound universal testing machine, and the beam deflections corresponding to known loads were obtained. A deflection of approximately 0.0001 inch occurred for each 500 pounds of load applied. A number of calibrations were made and a calibration curve was plotted. When used for measuring loads, it was only necessary to read the central deflection on the Ames dial and the corresponding load could be read from the curve.

DEFORMATION-MEASURING APPARATUS.—Deformations of the top of the slab were measured at right angles to the supports, and also, in the case of one slab, parallel to the supports, with a Berry strain gauge of 20-inch gauge length. The degree of accuracy attained was probably within 0.0002 inch in that gauge length. Short brass plugs drilled at one end with a No. 55 drill were embedded in the concrete, or in some cases cemented in holes drilled for the purpose; and the movements of these plugs as measured with the strain gauge were considered the fiber deformations.

In the last slab tested (No. 934) deformation readings were also taken of the steel reinforcement, and for this purpose holes were drilled in the steel bars 20 inches apart to accommodate the points of the strain gauge. Although readings were not taken on all of the bars, a sufficient number were measured to determine the distribution of the steel stresses throughout the slab. The layout of strain-gauge points between which readings were made is shown in figures 2, 3, and 4. The arrowheads mark the position of the points on the top of the slab and in the case of slab 934 (fig. 4) the gauge points in the steel are marked by small circles.

DEFLECTION-MEASURING APPARATUS.—The deflection measurements were made in somewhat different ways during these tests, and the apparatus was improved as the tests progressed. In its final form in slab 934, the deflection-measuring equipment consisted of a network of piano wires stretched tightly at a fixed distance above the concrete supports, and being entirely independent of the slab. At the points where measurements were taken, steel plates were set in plaster of Paris on top of the slab. Readings were then made between these plates and the wires by means of a specially designed instrument consisting of a brass stand carrying a bell-crank lever, one end of which touched on the piano wire above and the other end bore on the plunger of an Ames dial. By means of a slow-motion screw the end of the bell-crank lever was adjusted to touch the wire as indicated by an electric buzzer. The dial readings taken at different loads then indicated the deflections at the various points on the slab. This instrument is probably a more convenient form of measuring device than the ordinary inside micrometer and is accurate to 0.004 inch.

DESCRIPTION OF SPECIMENS

All three specimens were 32 feet wide, 16 feet span, and were made of machine-mixed concrete in the proportions 1 to 2 to 4. Potomac River sand and gravel were used as the aggregates, mixed with Portland cement. A rather wet mix was used, and the work of molding was done by laborers at the Arlington Farm who were experienced in work of this character. There was no attempt to make the concrete any better than it would ordinarily be made in the field, but efforts were

directed to secure work thoroughly representative of that obtained under field conditions. The sand was a good grade for use in concrete, and the gravel was clean, well graded, and free from weak pebbles.

The steel reinforcing consisted of ¾-inch plain square bars in slabs 835 and 930, and the bars in slab 934 were ½-inch square. The yield point of this material is about 39,000 pounds, and the ultimate strength 60,000 pounds per square inch.

The slabs were necessarily built in place on their supports, and the forms were struck at the end of about two weeks. The concrete was sprinkled daily for several weeks during the earlier stages of hardening and was allowed to cure protected from the weather until the destruction of the slab.

Table I contains the essential data concerning the slabs tested.

TABLE I.—*Description of reinforced-concrete slabs used in tests*[1]

Serial No.	Thickness.		Reinforcing.			Modulus of elasticity of concrete.	Central breaking load of slab.
	Total.	Effective.	Size.	Spacing.	Per cent.		
	Inches.	*Inches.*	*Inches.*	*Inches.*			*Pounds.*
835.....	12	10½	¾ (plain square).	10. 5	0. 75	2, 900, 000	119, 000
930.....	10	8½	¾ (plain square).	8. 87	. 75	4, 000, 000	80, 000
934.....	7	6	½ (plain square).	5. 56	. 75	3, 000, 000	40, 000

[1] The slabs were not reinforced transversely.

At the time the slab specimens were made, 8 by 16 inch concrete cylinders were molded from the same mixture and were allowed to cure under the same conditions as the slabs. These were tested later for their crushing strength and modulus of elasticity.

METHOD OF TESTING SLABS

At the age of 28 days the initial strain-gauge and deflection readings were taken with no load on the slab. The first load was then applied through an 8-inch cylindrical bearing block set in plaster of Paris at the center of the slab. Strain-gauge and deflection observations were made again over the entire slab. Due account was taken of the air and concrete temperatures in order to make corrections for any appreciable change occurring during the progress of the tests. The increments of load applied to the different specimens were varied in the different slabs, depending on their thickness, and the aim was to stress neither the steel nor the concrete beyond working limits, also to obtain about five increments of load within the working load.

After readings over the entire slab had been taken, check readings were made at various points; and invariably it was found that these check readings showed an increased deformation in the concrete even though its temperature remained constant. Moreover, upon releasing the load entirely it was found that considerable permanent deformation remained in the concrete. This phenomenon can be attributed only to the "flow" or gradual change in length of the concrete even when under small stresses and is significant, for it shows the importance of the time effect on the relation of stresses and strains in concrete. If the strain readings on the top of the slab, loaded for five or six hours, be used to estimate the stresses in the concrete, based on the initial modulus of elasticity of the concrete, this estimated stress will be greatly in excess of the true stress conditions.

In view of the fact that the deformations which take place in the concrete under a sustained load are continually increasing and remain partially permanent, and that the only deformations of value are those indicative of the stress, all of the final calculations and deductions are based upon results obtained by taking zero deformation readings just before applying the load. Deformations thus obtained by taking the difference between the strain-gauge readings at the zero load and the testing load (all within an hour or so), represent more accurately the elastic deformations and are a better indication of the stress existing in the concrete than those obtained from any initial or previous zero readings.

GRAPHICAL REPRESENTATION OF DATA AND RESULTS

A great amount of numerical data has been taken during the tests of these three concrete slabs. Some of these data were preliminary and served only to indicate methods and limits. Those data which have a direct bearing upon the problem are shown graphically in the accompanying curves (fig. 2–28).

FIGURES 2, 3, AND 4.—The layout of the points in the concrete and the steel over which the strain-gauge readings were taken are shown in figures 2, 3, and 4. In a few cases readings were made between all points, but in general only the readings along a center line (5–6) parallel to the supports were taken, as this gives sufficient data for determining the effective width. In all mention of strain-gauge or deformation readings it should be understood that they are measured between points on a line perpendicular to the supports, unless expressly stated to be otherwise.

FIGURE 5.—Figure 5 shows the variation of the concrete deformations for different concentrated center loads, along the center line of the slab. The ordinates of these curves are influenced slightly by the time factor or "flow" in the concrete; hence, the values for the effective width b are somewhat erratic in their relation to the load.

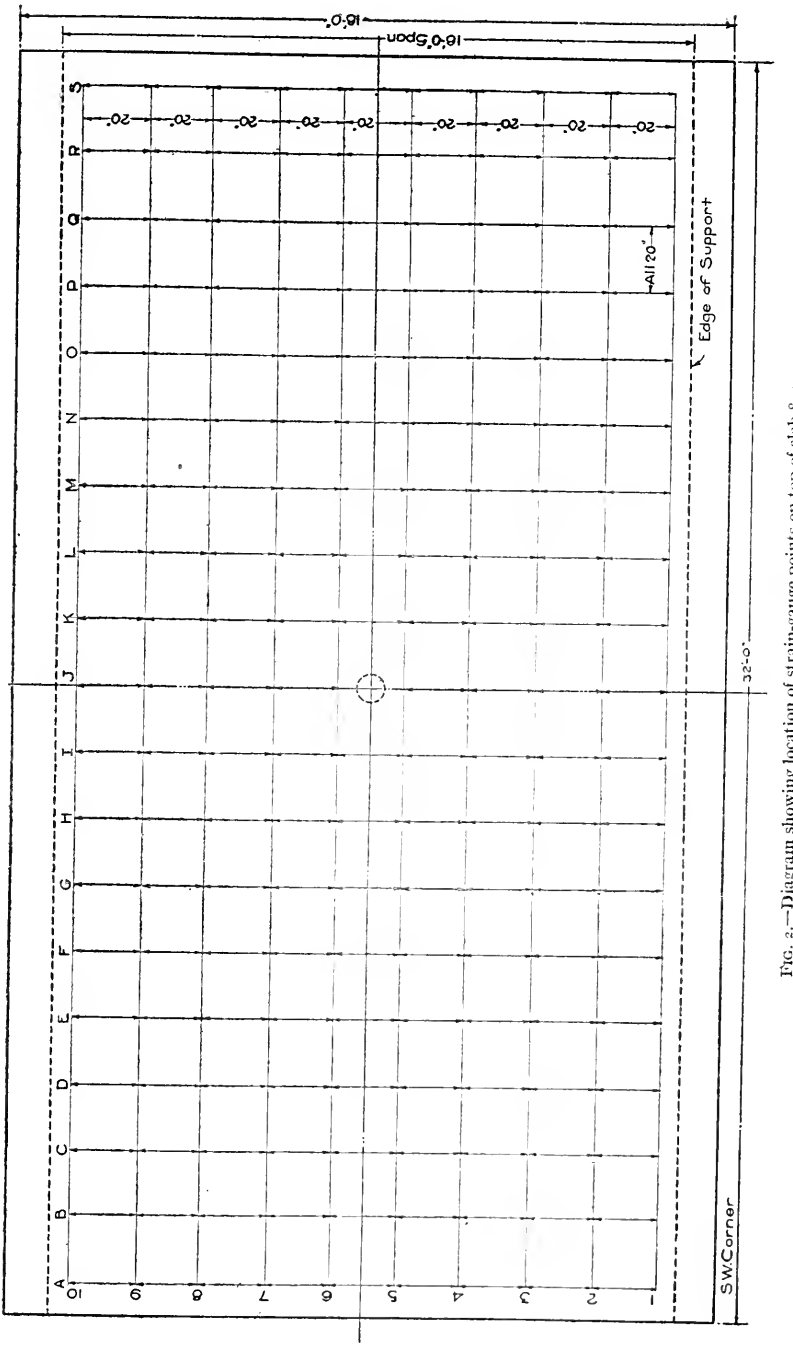


FIG. 2.—Diagram showing location of strain-gage points on top of slab 835.

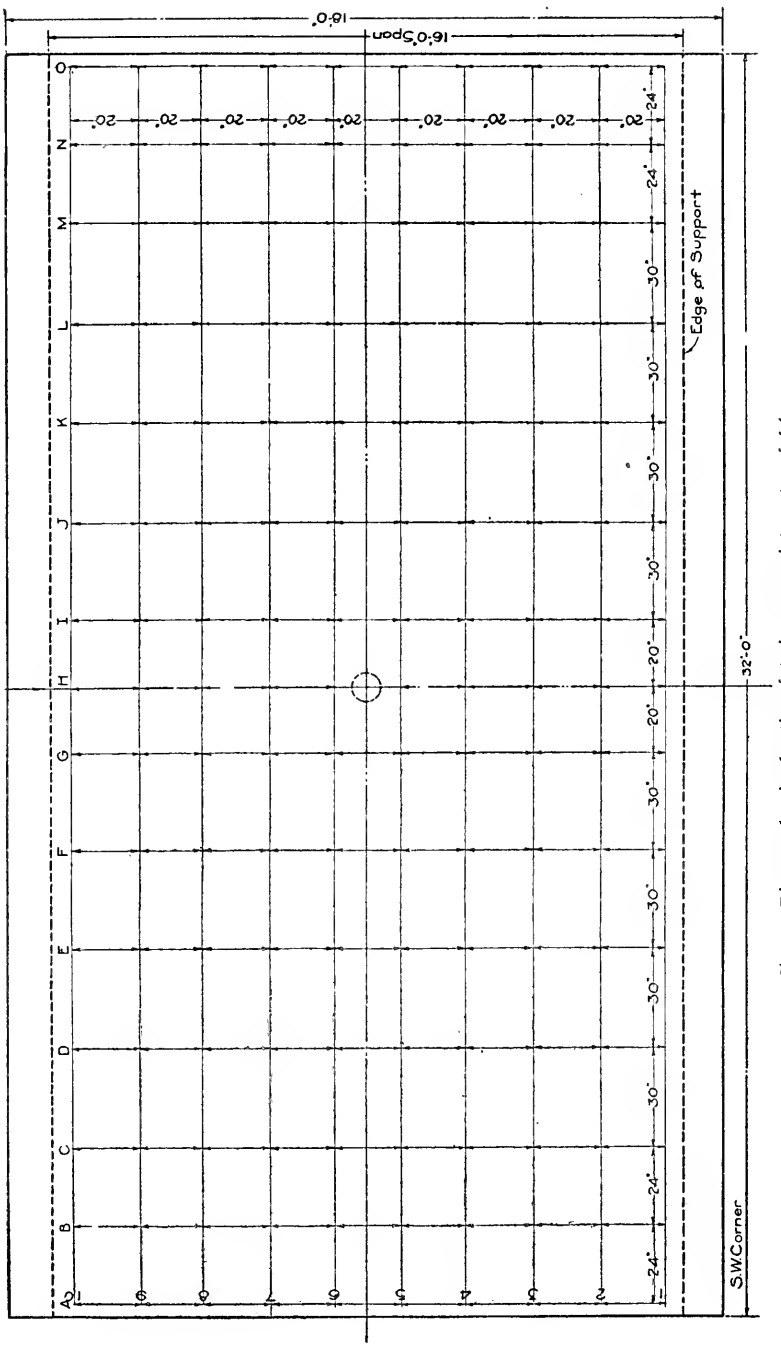


FIG. 3.—Diagram showing location of strain-gauge points on top of slab 930.

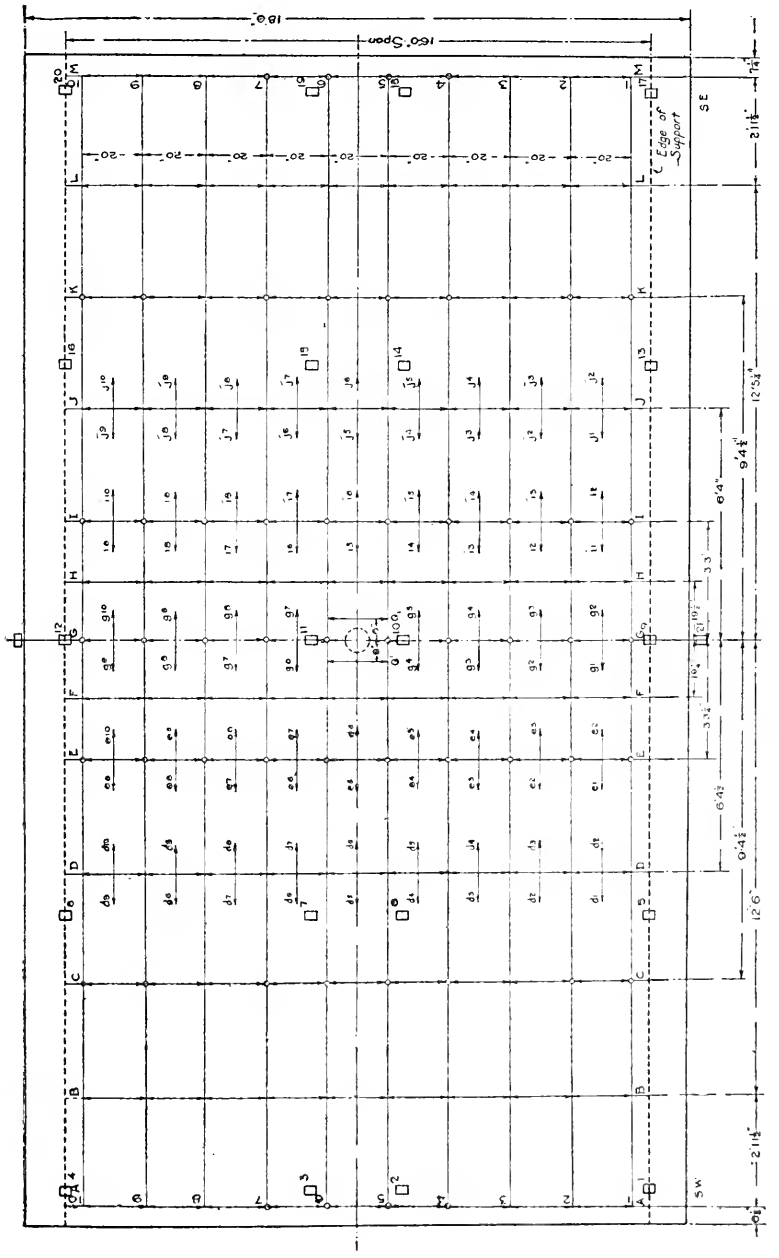


FIG. 4.—Diagram showing location of strain-gauge points on top and bottom of slab 934.

FIGURE 6.—Two curves, A and B, are shown here to indicate the deformations which resulted from the removal of the forms. The flow, or increase in the deformations, is about 80 per cent in three days. The curves C–D, E–F, G–H, I–J, and K–L show the large difference in the deformation and effective width values between those obtained by the use of a zero strain-gauge reading taken several weeks before, with several intervening loadings, and those obtained from a zero reading taken just before the loading. The data and results of curves C, E, G, I, and K are the only ones of value.

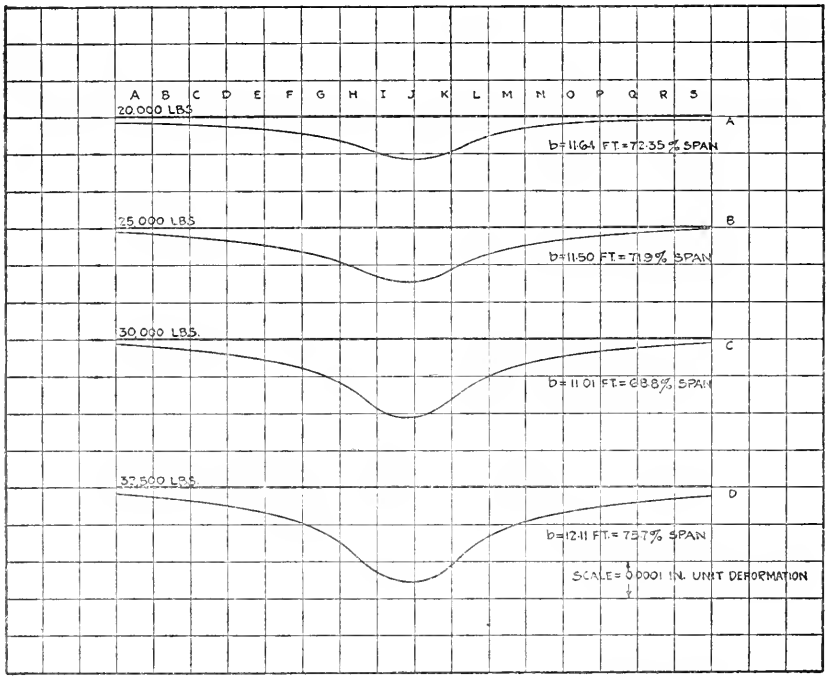


FIG. 5.—Concrete deformation curves for concentrated center load on slab 835.

FIGURE 7.—The difference between these curves shows the magnitude of the set, or permanent deformation, which may occur between two applications of the load, each loading having been applied immediately after a zero reading of the strain-gauge points, with 24 hours intervening between the loadings. The second application shows a smaller deformation than the first. This is true for both the concrete and the steel deformations. The effective widths are based upon the first application of the load.

FIGURE 8.—These curves are shown to emphasize the importance of considering the time factor and its effect upon the deformations in concrete structures. Curve 1 shows the immediate effect of the load. After about 5 hours the load was removed, then again applied 20 hours later

and allowed to remain on for two days, giving curves 2 and 3. The load was then removed, and curve 4 shows the amount of set about two hours later. This set is somewhat reduced after a few days' rest. The values of the effective widths shown in this figure differ very largely and are also indicative of the fact that the time factor is very important.

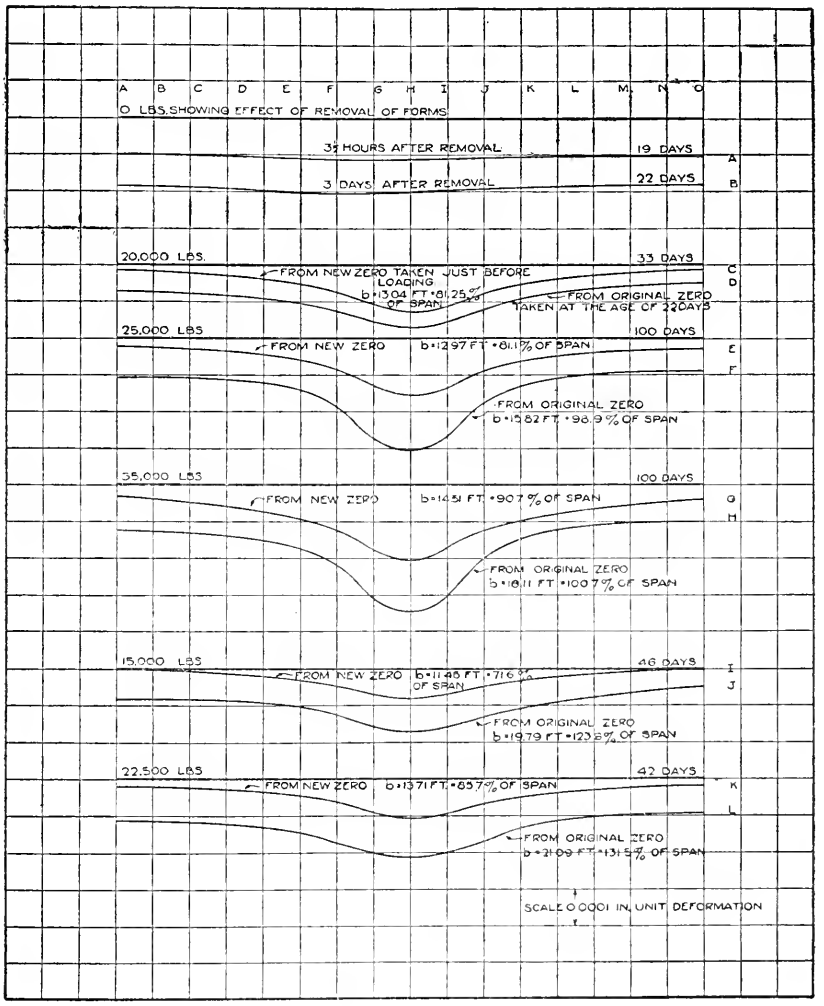


FIG. 6.—Concrete deformation curves for slab 930.

FIGURE 9.—Concrete deformations under 2-point loadings are shown for two-load values. The 40,000-pound load was applied immediately after taking the zero reading, and the deformations taken at once. The load was then increased to the 80,000-pound value and deformations again taken. The whole operation required not over two hours. The local effect at the load points is very pronounced for the larger load.

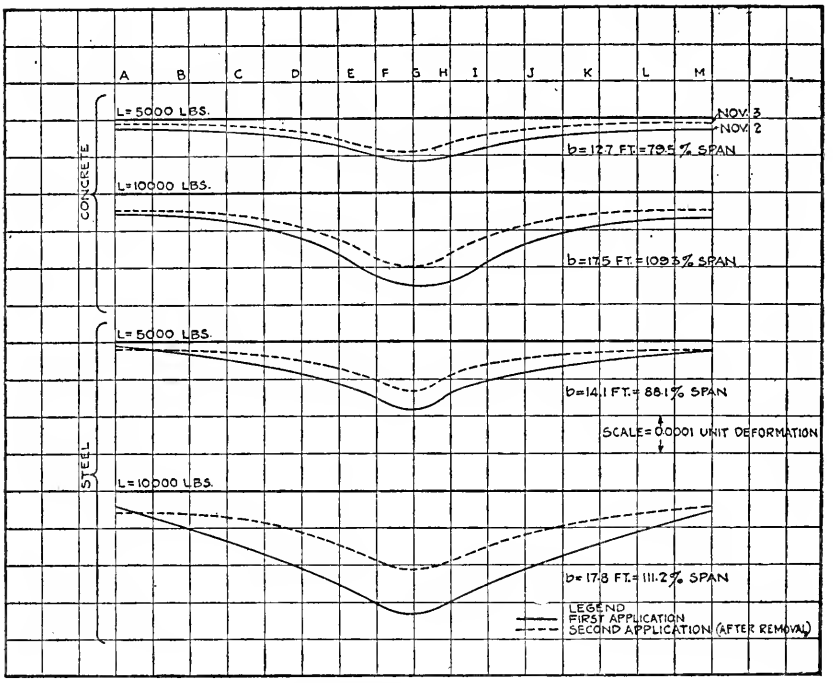


FIG. 7.—Deformation curves for slab 934.

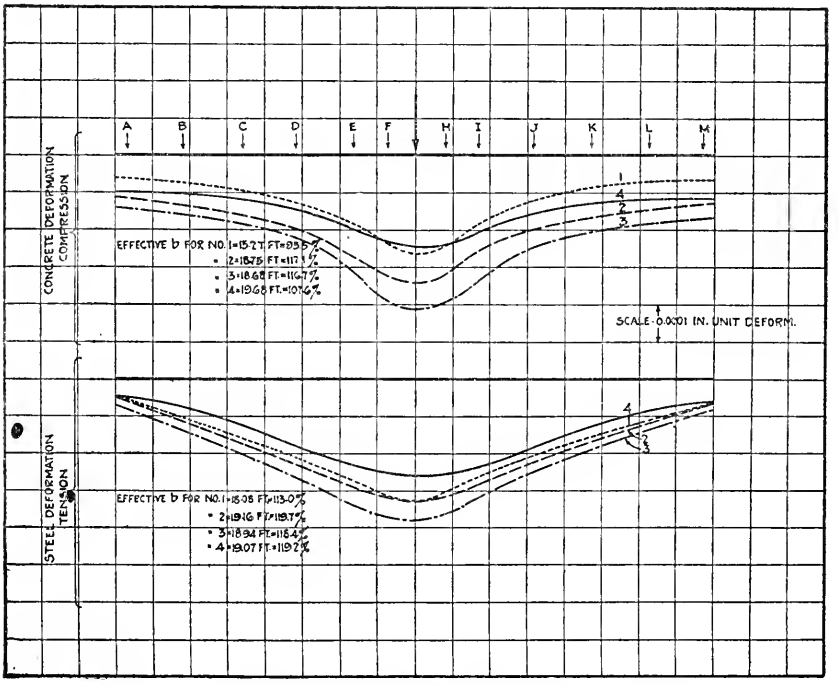


FIG. 8.—Deformation curves for slab 934, computed from first zero reading.

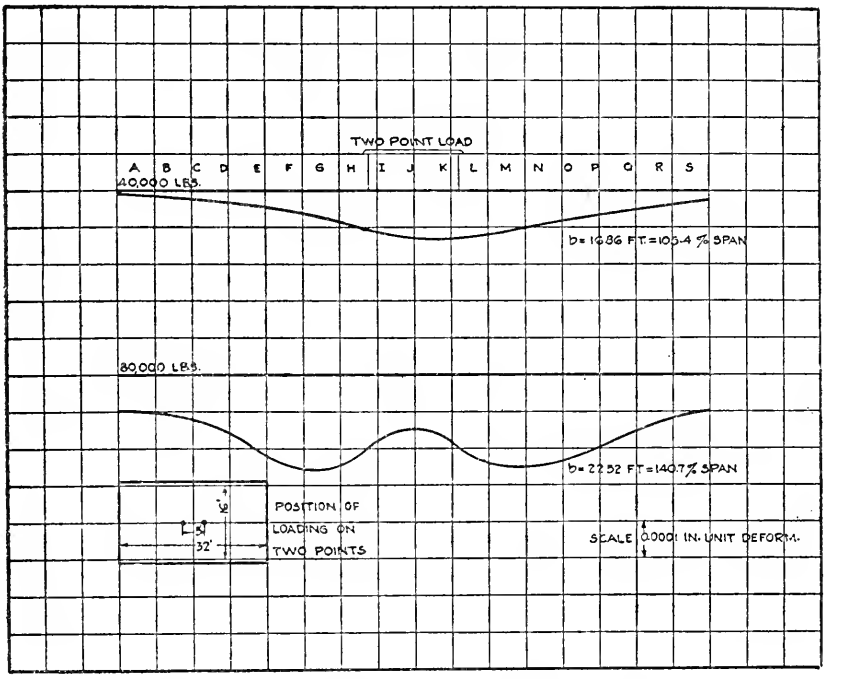


FIG. 9.—Concrete deformation curves for slab 835 with 2-point loading.

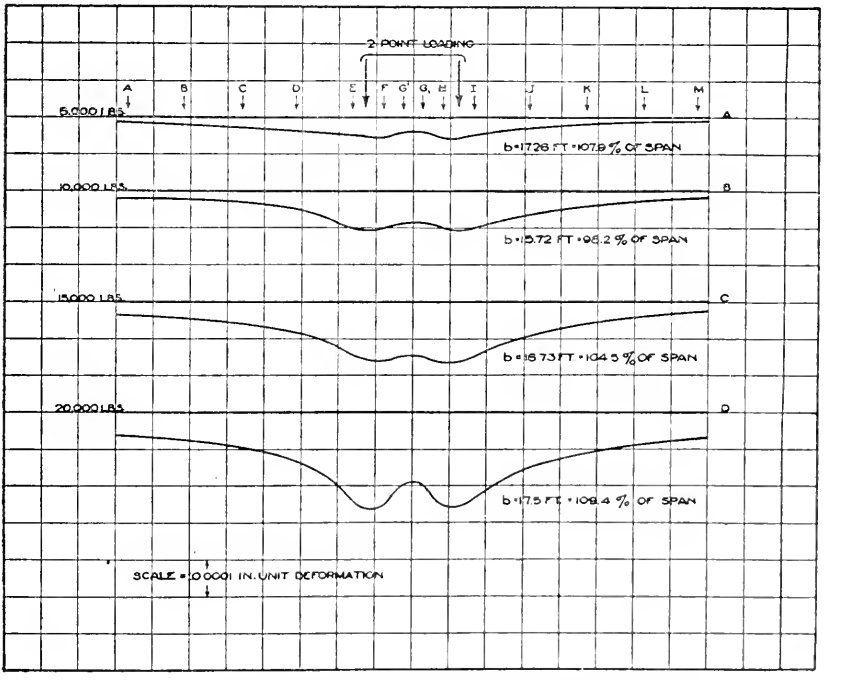


FIG. 10.—Concrete deformation curves for slab 934 with 2-point loading.

The effective width is not materially affected for the 40,000-pound load; but for the 80,000-pound load, which produces the working fiber stress, the effective width is very largely increased. .

FIGURES 10 AND 11.—The curves on these figures show a more pronounced local effect in the concrete at the load points than the same character of loading on the thicker slab. It should be noted that for the working load of 20,000 pounds the effective width for this 2-point loading is the same as for the single-point center loading.

FIGURES 12 AND 13.—The results for 4-point loading under different loads are shown in these curves for slabs 835 and 934. The effective

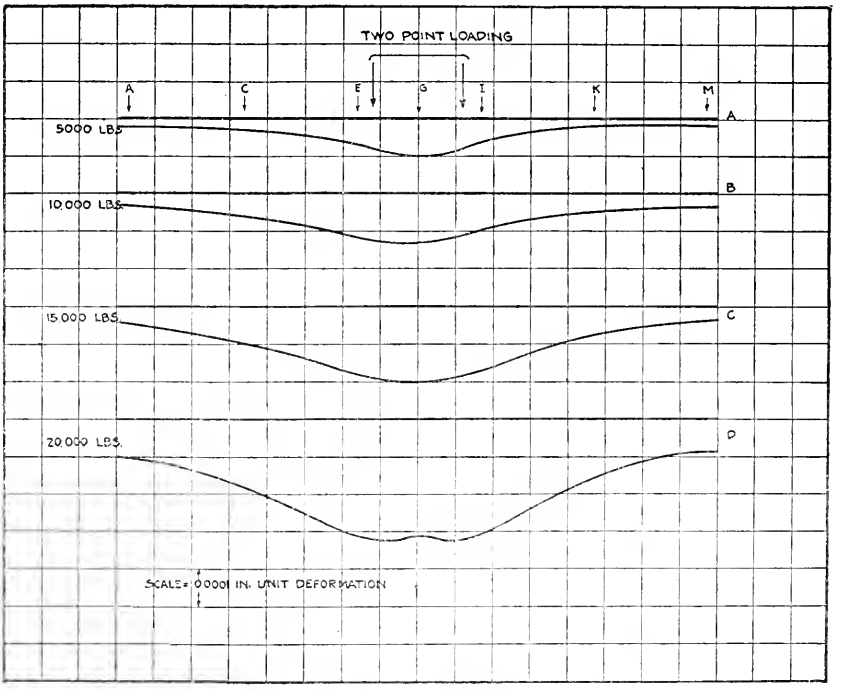


FIG. 11.—Steel deformation curves for slab 934 with 2-point loading.

width is materially affected by the width between the load points; it seems to be increased by not less than 56 per cent of the span length for slab 835, and 93 per cent for slab 934.

FIGURES 14, 15, AND 16.—The deflection data are shown on these figures. The curves are plotted to show the deflection values along a center strip parallel to the supports. In figure 14 curves have been plotted showing the flow and set in the slab under a sustained load and as effected by two applications. Two values for effective widths are shown, which have been obtained from the deflection curves in the same manner as from the concrete deformation curves described above; but these values should not be used in the design of slabs.

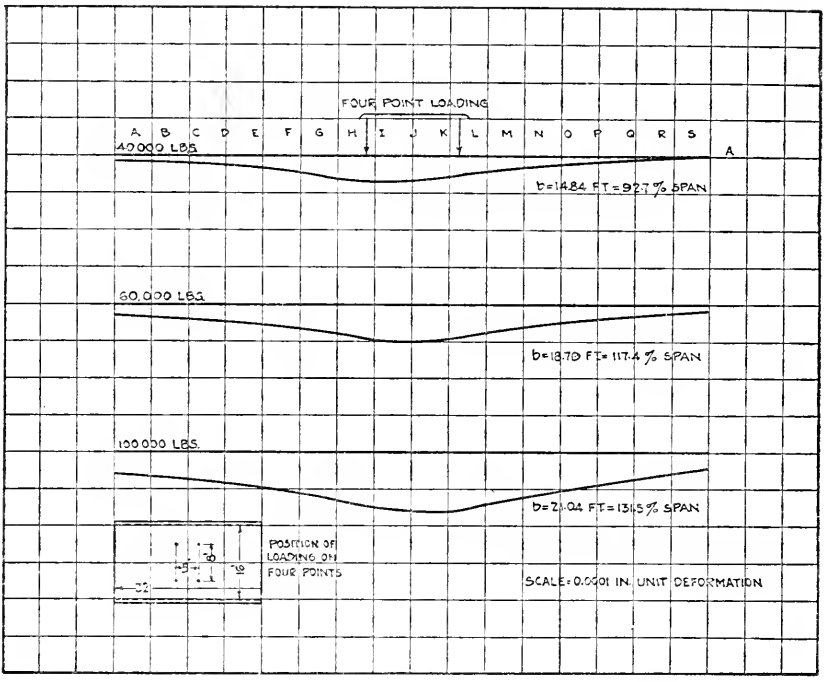


FIG. 12.—Concrete deformation curves for slab 835 with 4-point loading.

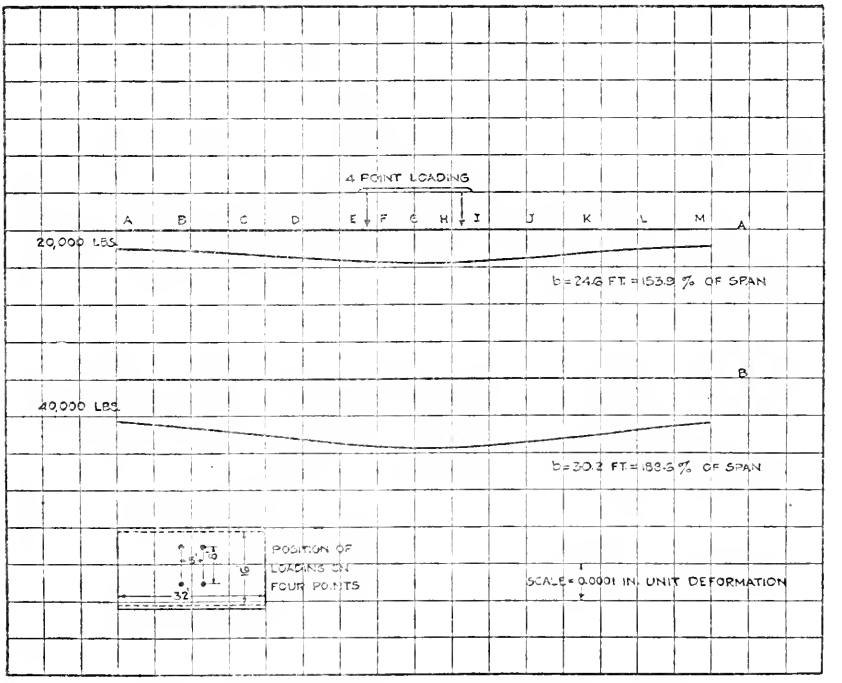


FIG. 13.—Concrete deformation curves for slab 934 with 4-point loading.

FIGURES 17, 18, AND 19.—After each slab was broken the cracks in the top and bottom were drawn to scale. The heavy full lines forming an approximate circle or ellipse around the load point are the tension cracks on the top of the slab caused by the overhang of the ends, after a large center deflection, at about breaking load. The remarkable symmetry of

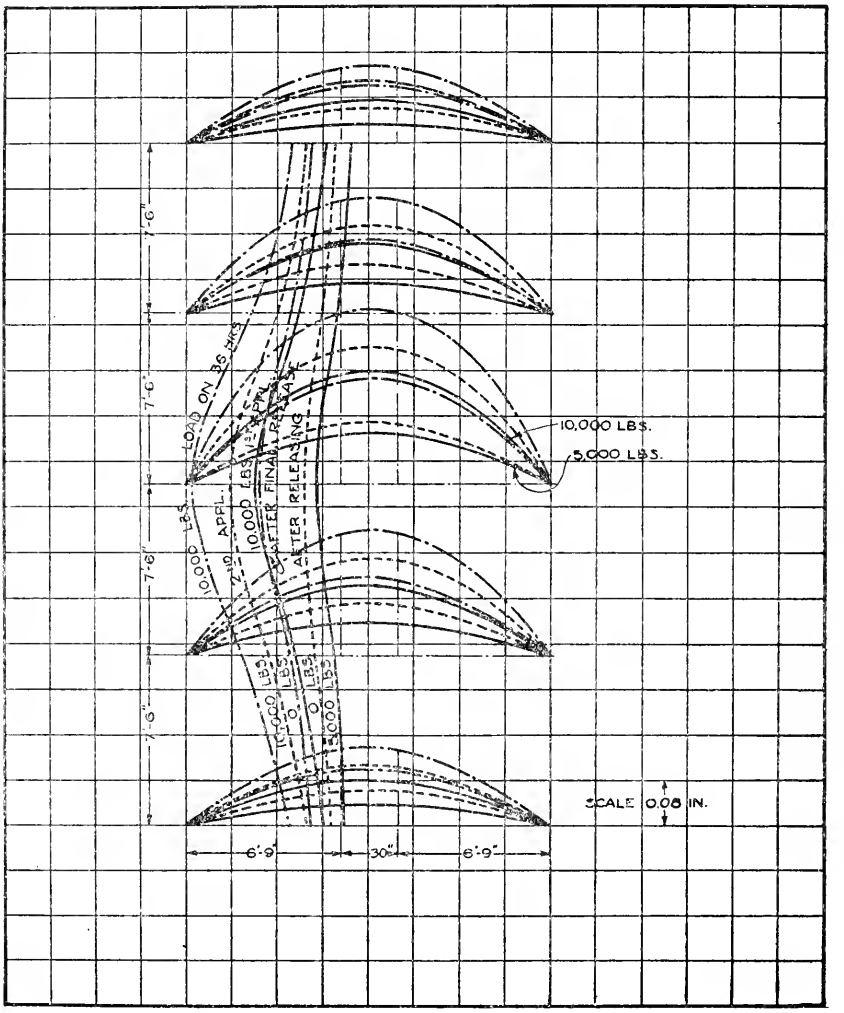


FIG. 14.—Deflection curves for slab 934 on first application of load.

these cracks is worthy of notice. There seems to be no definite relation between the effective width at working loads and the width over which the cracks extended at failure; in fact, it is hardly reasonable that there should be any definite relation, for one case is dealing with safe working stresses within the limit of elasticity, and the other with breaking loads.

Table II shows the breaking loads and their relation to the depth of the slab. Note that the breaking loads are almost directly proportional to the squares of the depths.

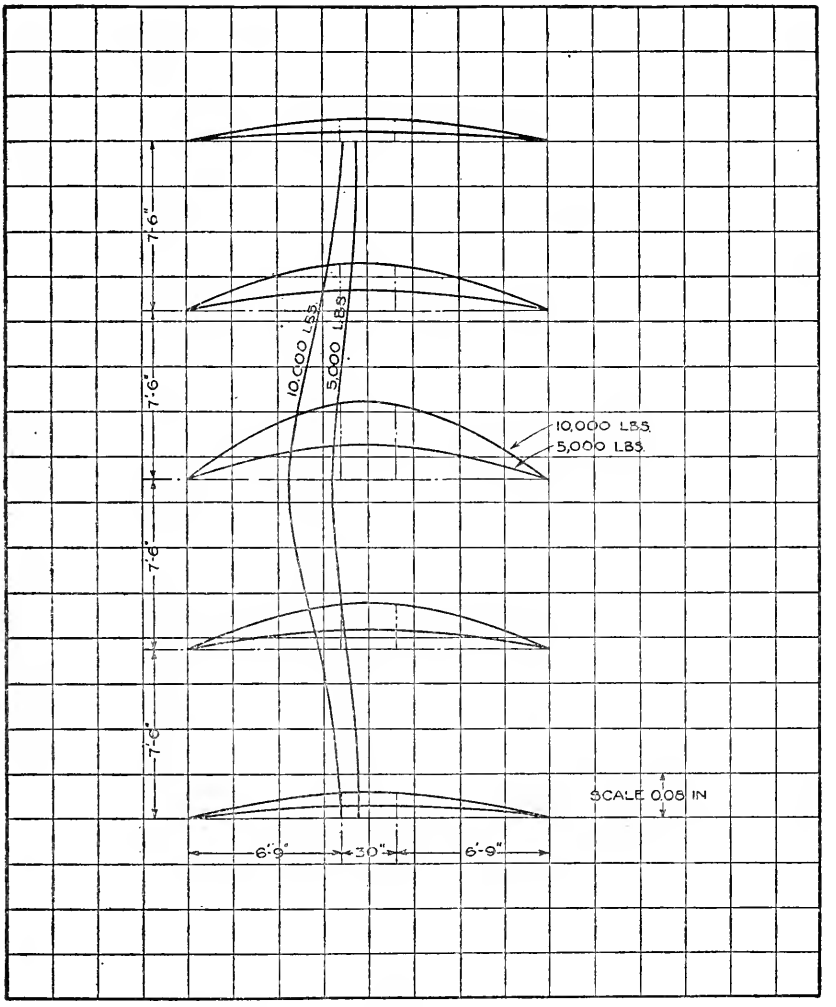


FIG. 15.—Deflection curves for slab 934 on second application of load.

TABLE II.—*Breaking loads of reinforced-concrete slabs and their relation to the depth of slab*

Serial No.	Effective thickness, d.	d^2	Breaking load.	Relations.	
				d^2	Loads.
835	10½	110.25	119,000	3.06	2.98
930	8½	72.25	80,000	2.01	2.00
934	6	36.00	40,000	1.00	1.00

STRESS DISTRIBUTION OVER THE WHOLE SLAB

For the purpose of determining the distribution of stress over the top of the whole slab, deformation readings at right angles to each other were taken on slab 934 for a working load of 10,000 pounds concentrated at the center.

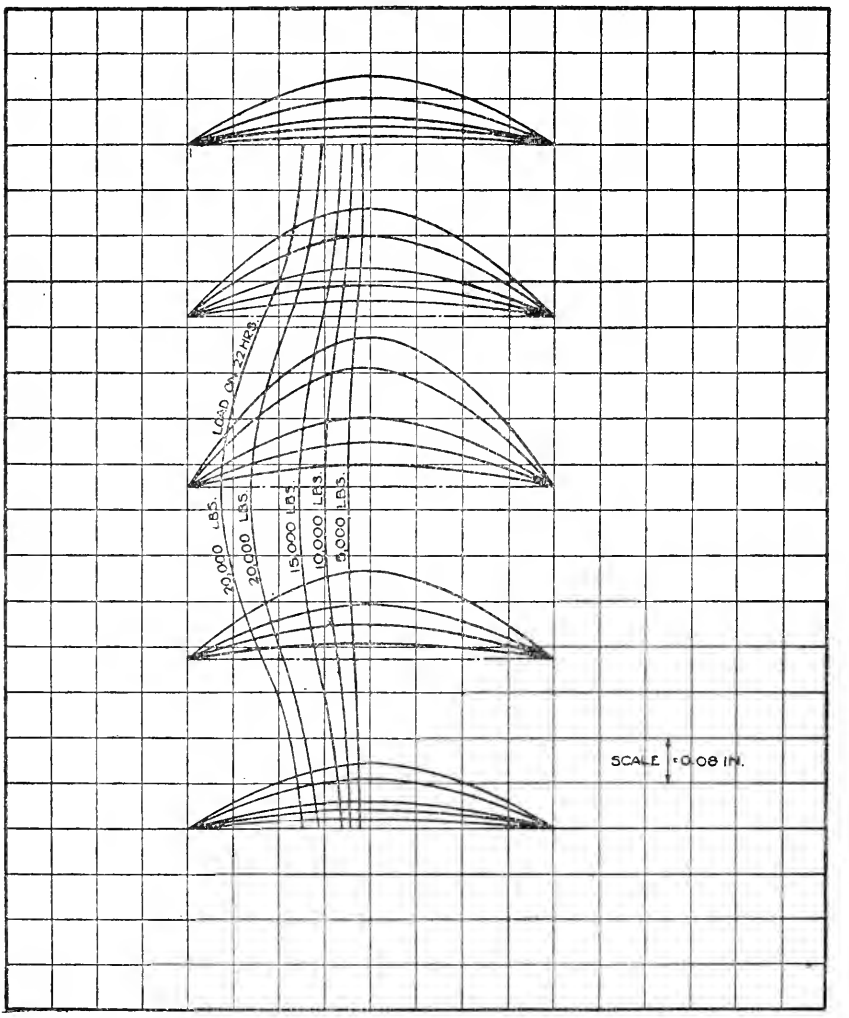


FIG. 16.—Deflection curves for slab 934 with 2-point loading.

FIGURES 20 AND 21.—The deformations measured perpendicular to the supports and plotted on base lines parallel to the supports are shown in figure 20. These curves show the variation of deformations along lines parallel to the supports. The same deformations plotted on base lines perpendicular to the supports, to show the variation in that direction,

are plotted on figure 21. Each curve as shown is an average of the plotted points. The light vertical lines serve only to locate each curve with its base line.

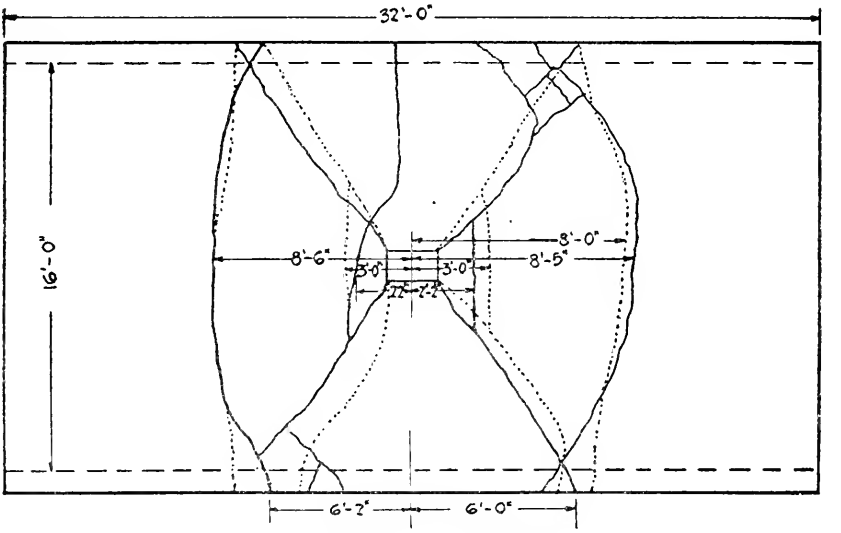


FIG. 17.—Diagram showing effect of breaking load on slab 835.

The variation of the distribution along lines parallel to the supports is is somewhat gradual and does not show any sudden changes; but the

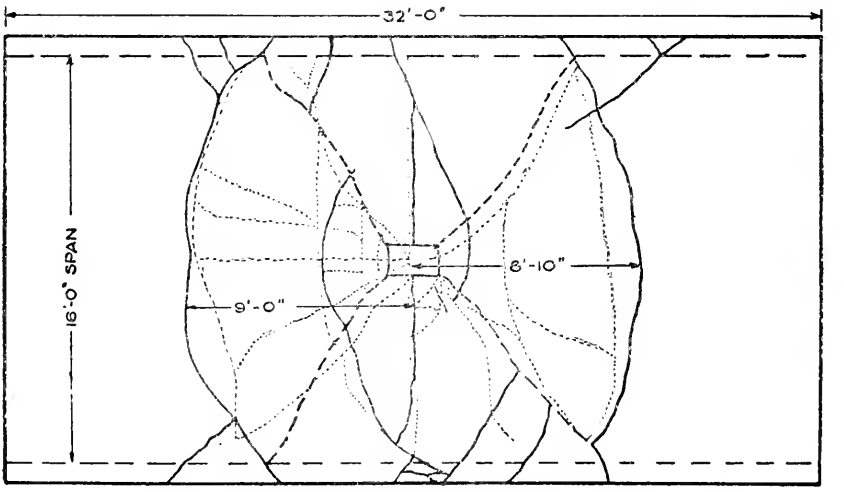


FIG. 18.—Diagram showing effect of breaking load on slab 930.

variation across the span near the center of the slab becomes somewhat critical at and near the load point, and this was more pronounced in the concrete than in the steel. (The steel data are not shown.)

FIGURE 22.—Lateral strain-gauge readings were taken on points parallel to the supports over the middle third of the slab, and these are plotted on base lines both parallel and perpendicular to the supports. The groups of closely drawn parallel lines serve only to connect each curve with its base line. Compression values of the deformations are plotted either to the left or below the base lines, and to the right or above, for values of tension in the concrete. The variations in these lateral deformations are the reverse of those of the longitudinal deformations shown in figures 20 and 21; they are more critical along lines parallel to the supports.

FIGURE 23.—The data of the last three figures have been collected and plotted as "iso-deformation lines," giving a series of lines or contours

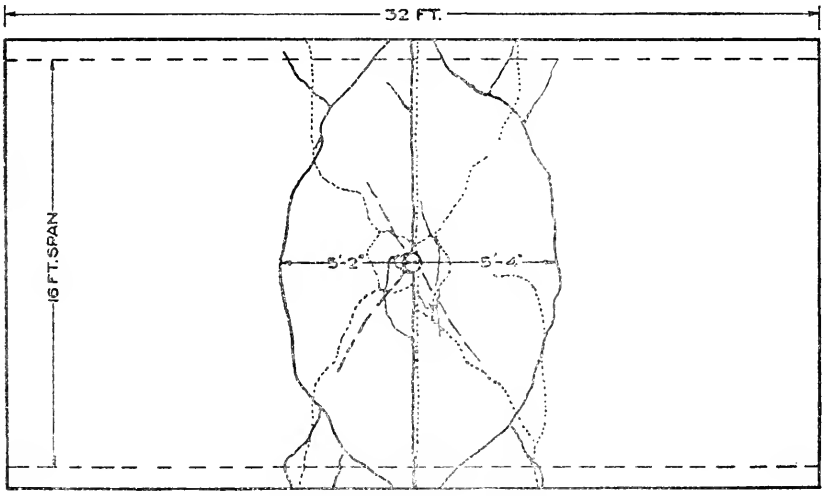


FIG. 19.—Diagram showing effect of breaking load on slab 954.

which represent equal deformations in the concrete on the top of the slab. The lines, as drawn, are averages of the plotted points. Figure 23 (also fig. 26) is more for academic interest and should be of service in the theoretical consideration of stress distribution.

FIGURES 24 AND 25.—These figures are similar to figures 20 and 21, and are plotted in the same manner, except that they represent the distribution of deformations under a working load of 40,000 pounds applied at four points. No lateral deformation readings are shown. The load points are indicated in figure 25. The local effect at the loading points is very pronounced.

FIGURE 26.—The data of the last two figures mentioned have been here collected and show the "iso-deformation lines" for the 4-point loading of 40,000 pounds, total. (See description of figure 23.)

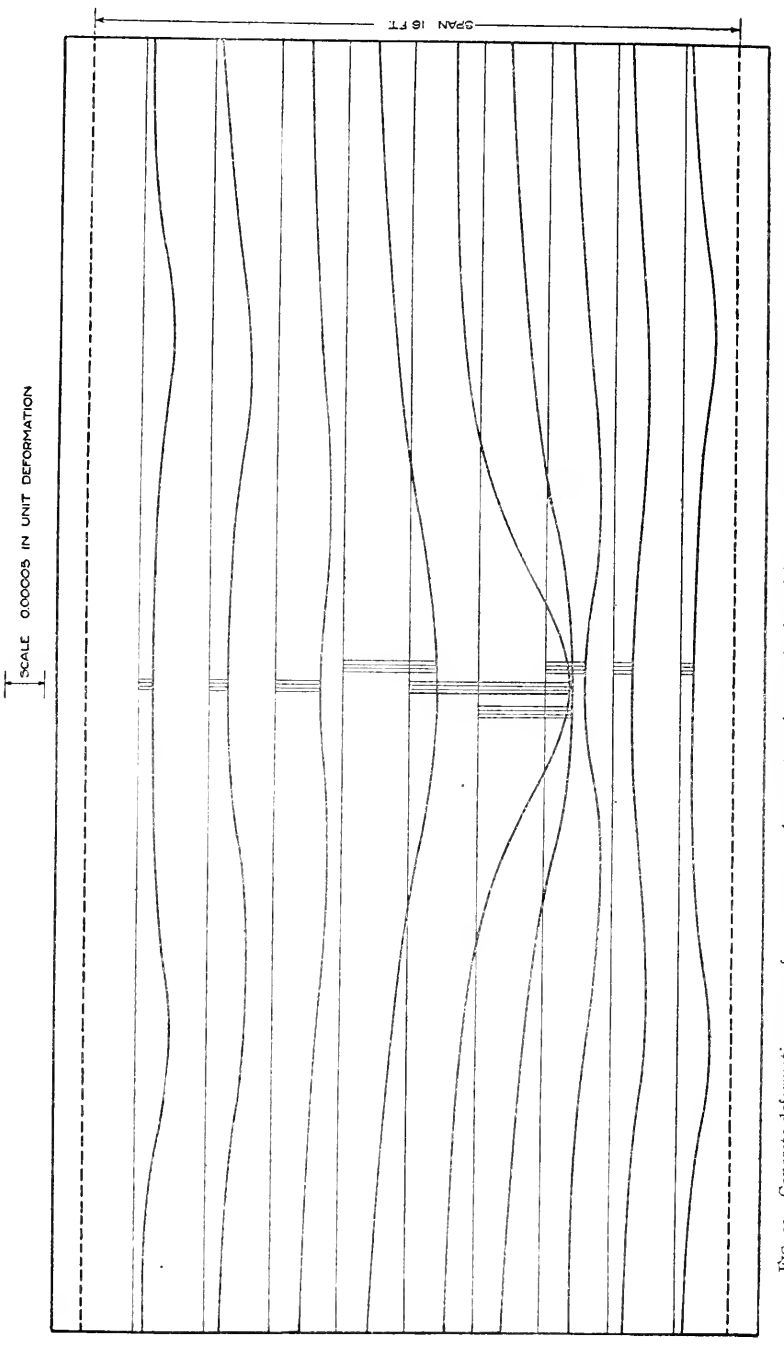


FIG. 20.—Concrete deformation curves for a 10,000-pound concentrated center load on slab 934. Variation in deformations plotted parallel to supports.

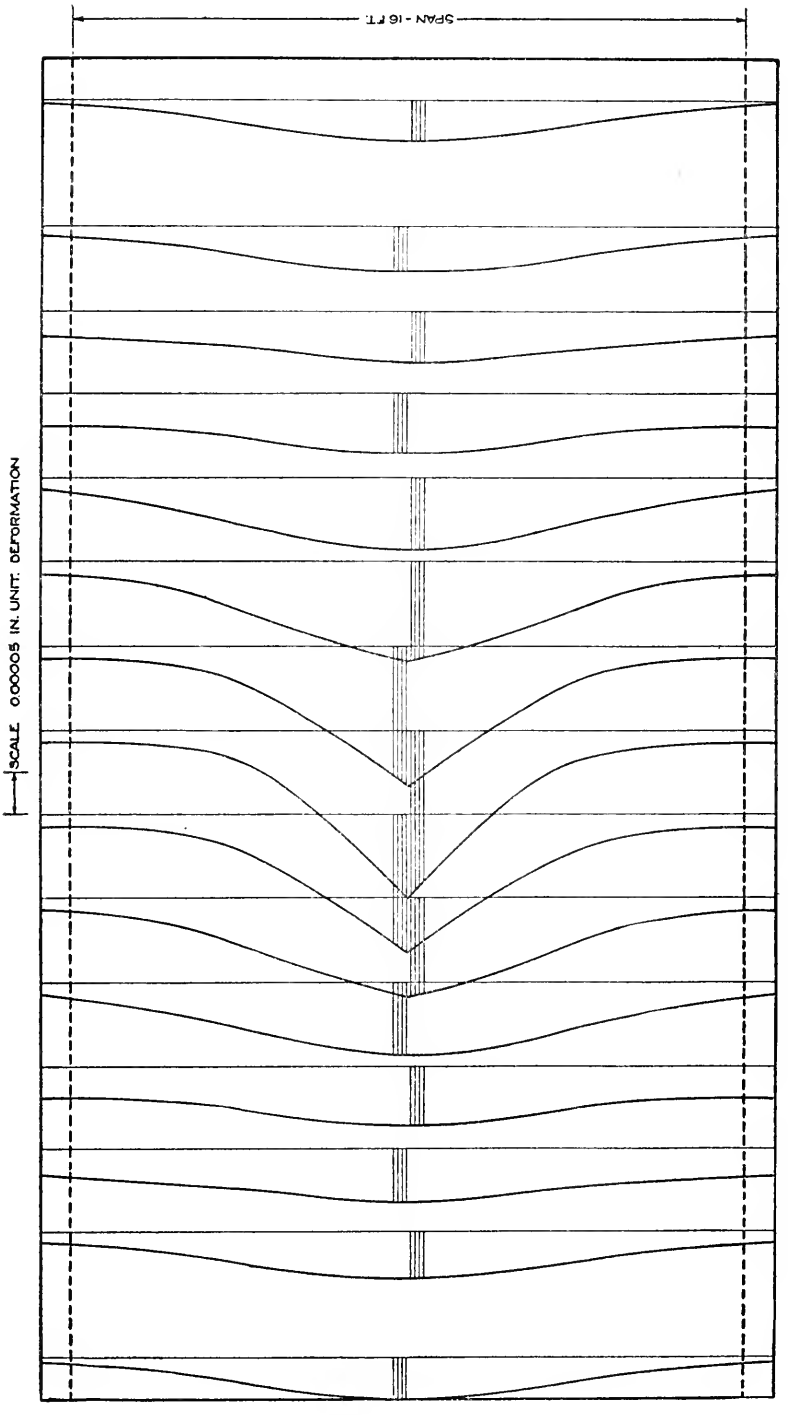


FIG. 21.—Concrete deformation curves for a 10,000-pound concentrated center load on slab 934. Variation in deformations plotted perpendicular to supports.

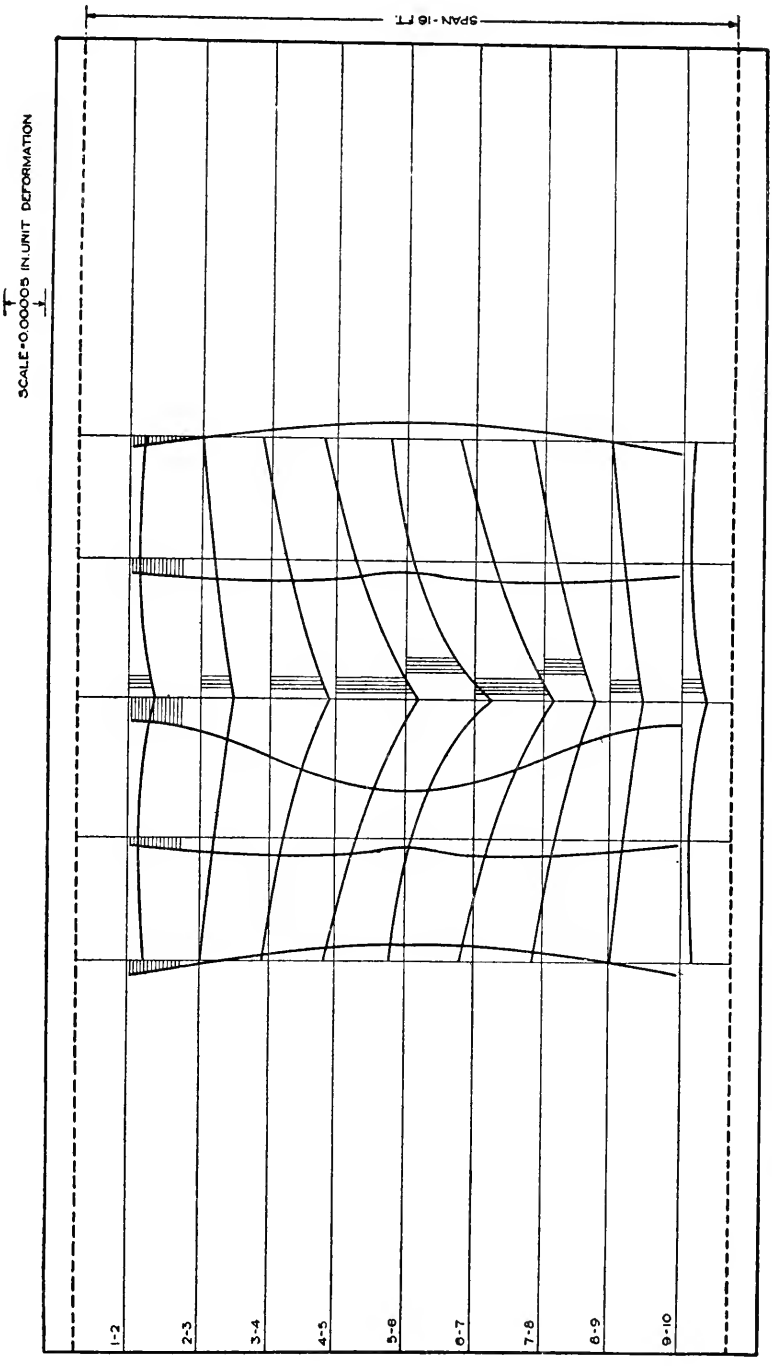


FIG. 22.—Concrete deformation curves for slab 934. Lateral deformations plotted both parallel and perpendicular to supports.

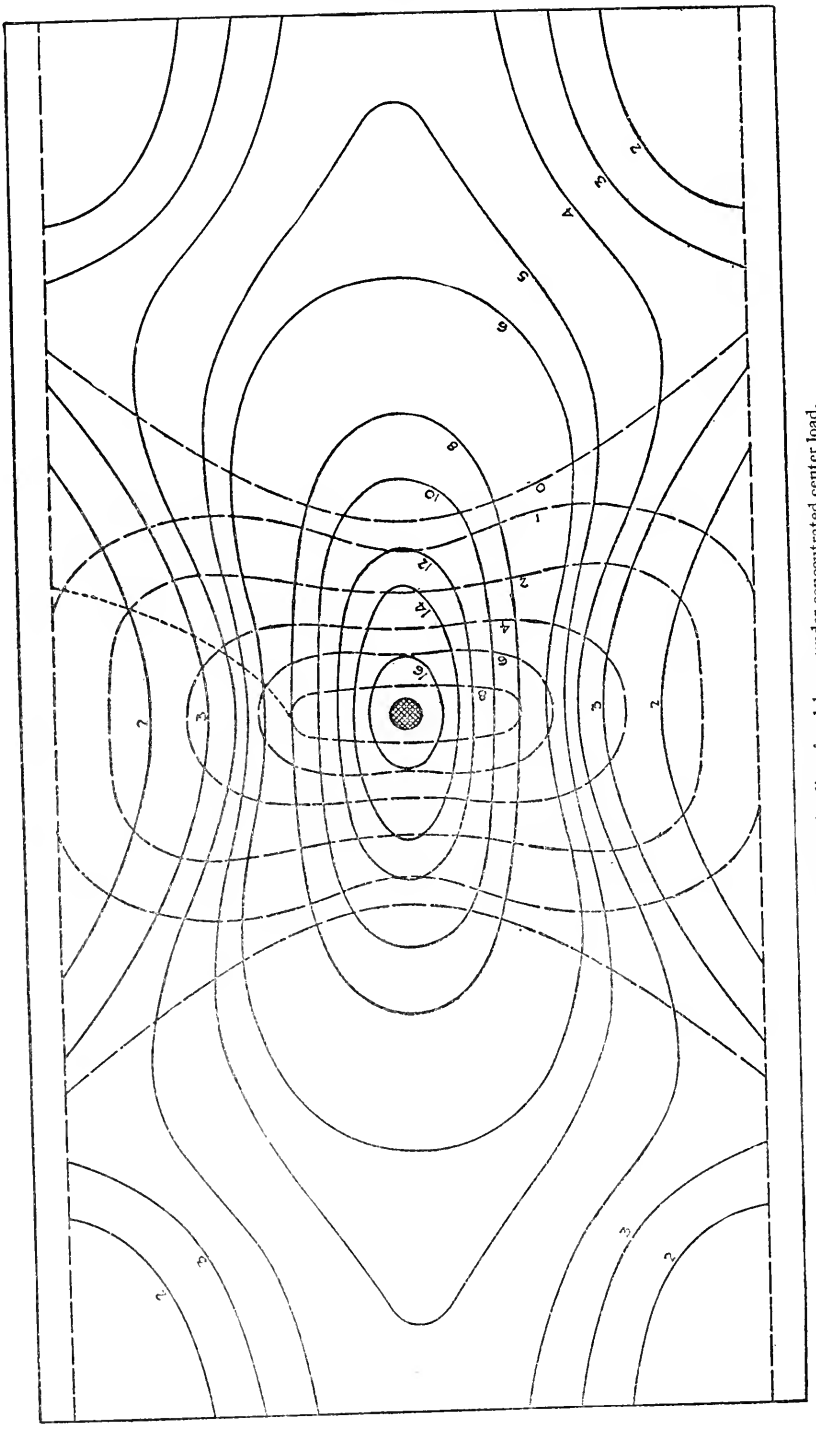


FIG. 23.—Iso-deformation lines for slab 934 under concentrated center load.

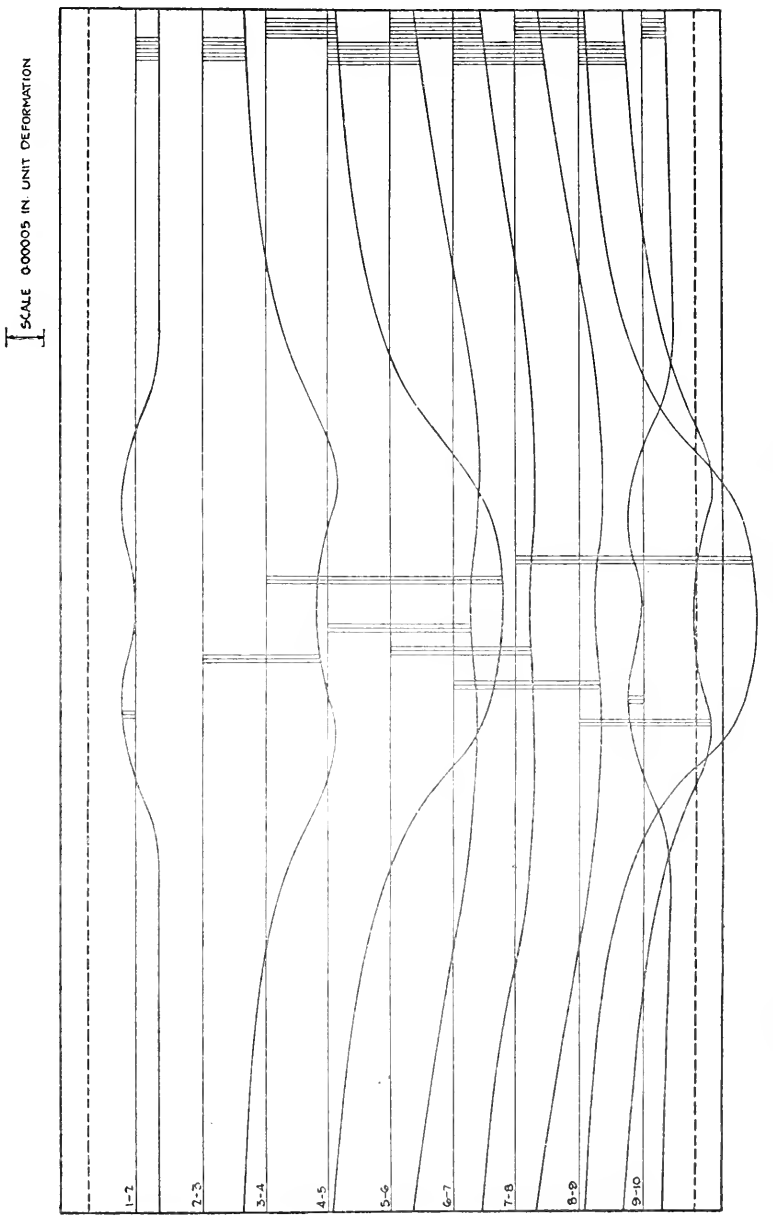


FIG. 23.—Concrete deformation curves for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports. Variation of deformations plotted parallel to the supports.

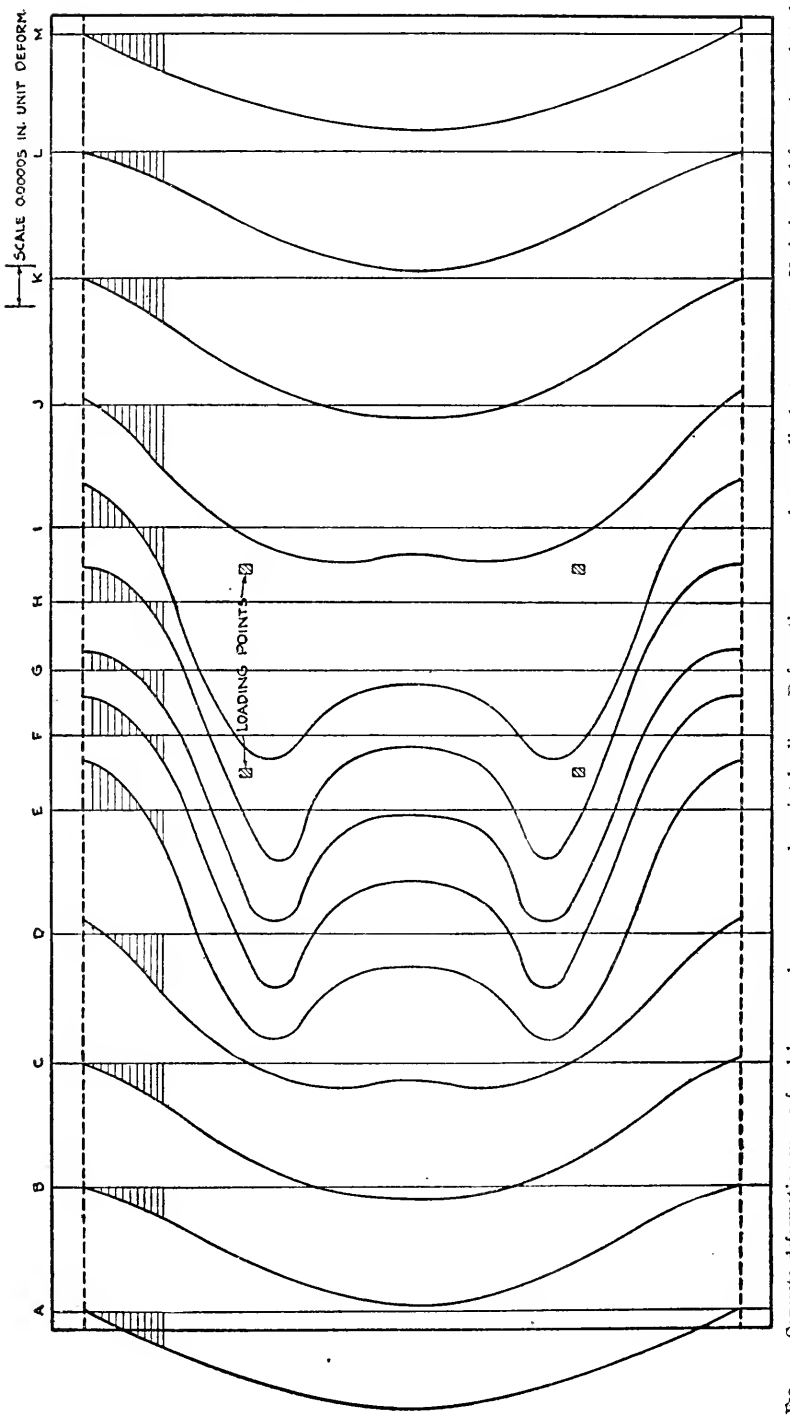


FIG. 25.—Concrete deformation curves for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports. Variation of deformations plotted perpendicular to supports.

CONCLUSION

If figure 27 is referred to, the influence on the effective width of the magnitude of the load and the manner of interpreting the results may be

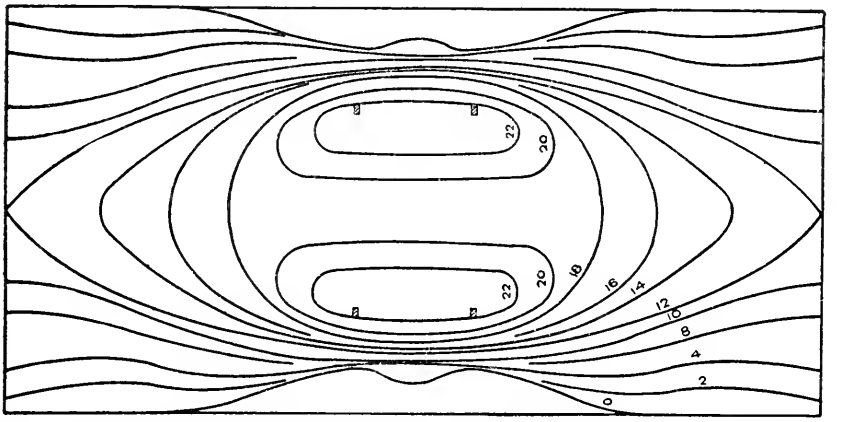


FIG. 26.—Iso-deformation lines for slab 934 under 40,000-pound 4-point loading. Deformations measured perpendicular to supports.

seen. It has been pointed out that the correct method of obtaining deformations is to base all calculations on zero readings taken just before

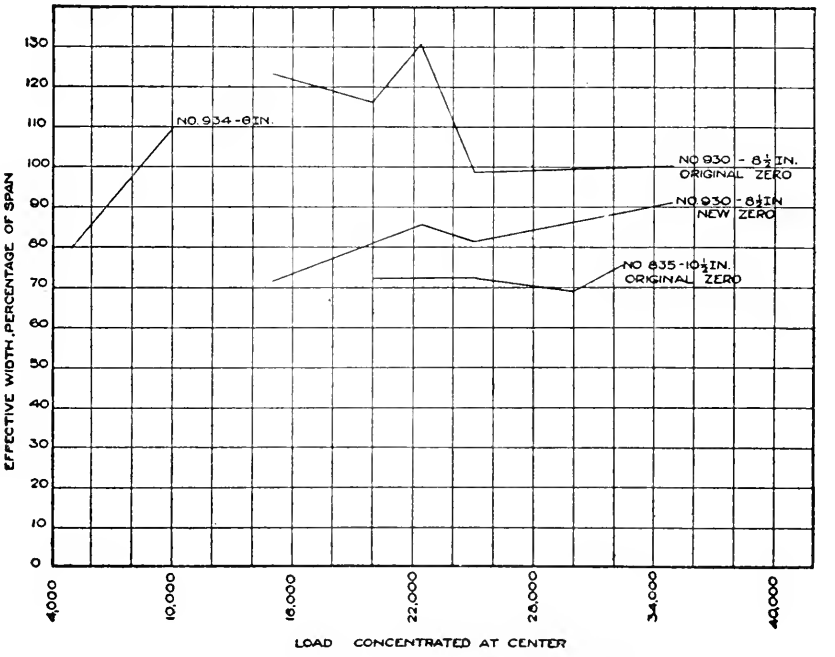


FIG. 27.—Curves showing effective width versus load (concentrated center load).

the load has been applied (designated on the curve as "new zero"). In the case of slab 930, figure 8, note the difference in effective width obtained

depending on the manner of considering the zero readings. The more conservative values are obtained by basing the calculations on the "new zero" readings, as was done in the case of slabs 930 and 934. Note that with an increase in load, the effective width seems to increase slightly. Values for effective width were obtained from the steel deformations, as well as from the concrete deformations, but it was found that the concrete deformations gave the most conservative widths, and these were therefore plotted.

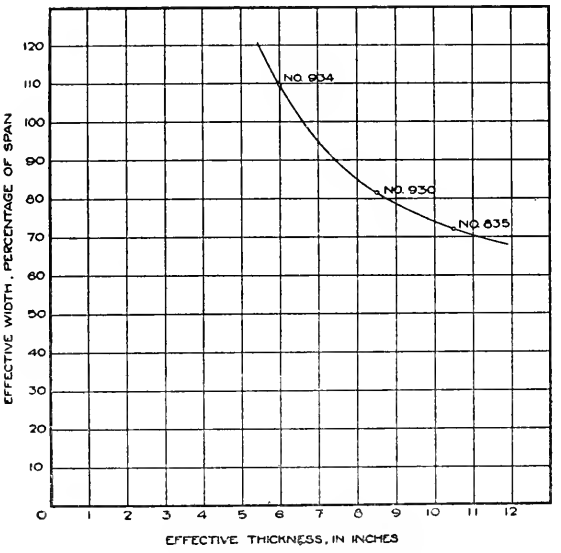


FIG. 28.—Curve showing effective width versus thickness.

In figure 28 the effect of variation in thickness of slab on effective width may be seen. Note that as the thickness increases, the effective width decreases, varying from 109 per cent of the span length for a 6-inch slab to 75 per cent of the span for a 10½-inch slab. The least value for effective width shown by these tests is roughly, then, about 0.7 of the span length. Judging from the curve of variation, it would seem that under extremely heavy loads, requiring very thick slabs, the effective width might be decreased as low, possibly, as 0.6 of the span length. However, 0.7 of the span will always be safe, and in general is a sufficiently conservative figure to use.

TABLE III.—*Effective widths of reinforced-concrete slabs, 16-foot span by 32 feet wide, for center loading*

Center load.	Slab 835 (10¼ inches effective thickness).	Slab 930 (8¼ inches effective thickness).	Slab 934 (6 inches effective thickness).
Pounds.			
15,000	.....	11.4 feet=71.6 per cent of span.	12.7 feet=79.5 per cent of span.
20,000	11.6 feet=72.3 per cent of span.	13.0 feet=81.2 per cent of span.	17.5 feet=109.3 per cent of span.
25,000	11.5 feet=71.9 per cent of span.	12.9 feet=81.1 per cent of span.	.....
32,500	12.1 feet=75.7 per cent of span.	.....	.....
35,000	.....	14.5 feet=90.7 per cent of span.	.....
Safe load	12.1 feet=75.7 per cent of span.	12.9 feet=81.1 per cent of span.	17.5 feet=109.3 per cent of span.

APPLICATION OF RECTANGULAR-BEAM THEORY TO DESIGN OF SLABS UNDER CONCENTRATED LOADS

The usual rectangular-beam design formulas may be applied to the design of slabs by merely substituting for b its value as determined by these investigations, $b=0.7L$. The corresponding formulas then become—

	FOR RECTANGULAR BEAMS	FOR SLABS UNDER CENTRAL CONCENTRATED LOADS
(1)	$M_c=\frac{1}{2}f_c kjbd^2$	$M_c=\frac{1}{2}f_c kj\frac{7}{10}Ld^2$
(2)	$M_s=pf_s jbd^2$	$M_s=pf_s j\frac{7}{10}Ld^2$
(3)	$p=\frac{a_s}{bd}$	$p=\frac{10a_s}{7Ld}$
(4)	$p=\frac{1}{\frac{f_s}{f_c}\left(\frac{f_s}{nf_c}+1\right)}$	$p=\frac{1}{\frac{f_s}{f_c}\left(\frac{f_s}{nf_c}+1\right)}$
(5)	$k=\sqrt{2pn+(pn)^2}-pn$	$k=\sqrt{2pn+(pn)^2}-pn$

It is interesting to note that in substituting for M_c and M_s in formulas 1 and 2 their value $\frac{PL}{4}$, the L's cancel, showing that the safe load-carrying capacity of the slab is independent of the span; thus—

1 becomes $\frac{PL}{4}=\frac{1}{2}f_c kj\frac{7}{10}Ld^2$ or $P=\frac{7}{5}f_c kjd^2$

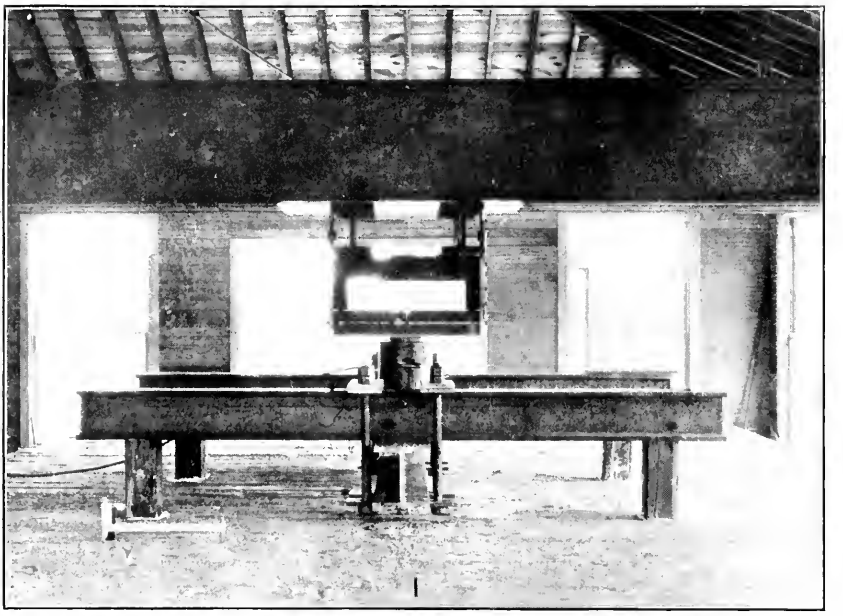
2 becomes $\frac{PL}{4}=pf_s j\frac{7}{10}Ld^2$ or $P=p\frac{14}{5}f_s jd^2$

The above investigations were made on slabs the width of which was twice the span length, so that the stress at the extreme edges was very small. The conclusions must therefore be applied to such cases only. When the ratio of width of slab to span length is less than 2, these conclusions may or may not apply, and additional investigations are now being made to determine the proper value of effective width to use under such conditions.

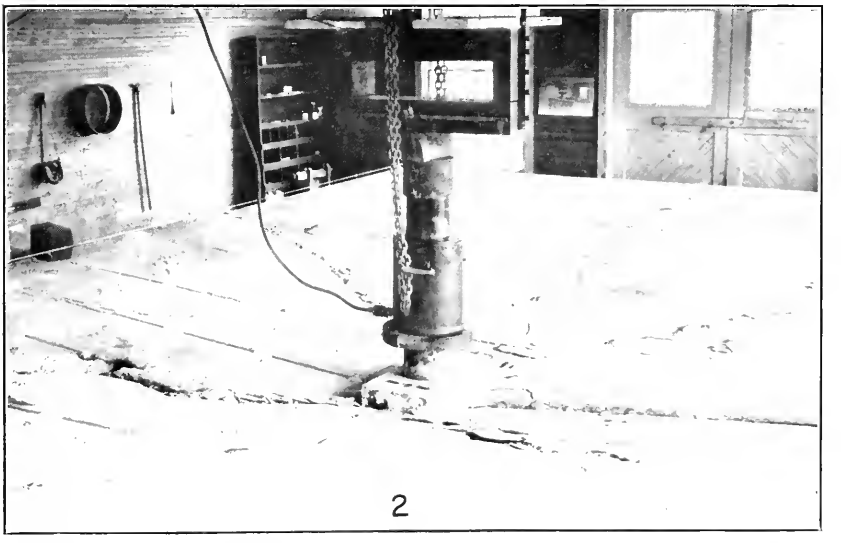
PLATE XXVI

Fig. 1.—Load-applying and load-measuring apparatus for testing reinforced-concrete slabs, showing set-up for 4-point loading.

Fig. 2.—Load-measuring apparatus and hydraulic jack for testing reinforced-concrete slabs.



1



2

OCCURRENCE OF STERILE SPIKELETS IN WHEAT

By A. E. GRANTHAM, *Agronomist, Delaware Agricultural Experiment Station*, and FRAZIER GROFF, *Student, Delaware College*

INTRODUCTION

The average spike of wheat (*Triticum* spp.) contains from 15 to 20 spikelets, each of which under favorable conditions is capable of producing two or more kernels. Ordinarily, however, the lower two or three spikelets on the spike do not develop. The only indications of their absence are the joints or nodes of the rachis which are thus exposed (Pl. XXVII). Hunt states that often in the cultivated varieties and always in the wild species the lower one to four are sterile. In this paper the term "sterile spikelet" is used to designate those spikelets at the base of the spike which for some reason fail to develop and produce seed. No account was taken of the sterile florets which might occasionally occur within the spikelet. The absent spikelets, as shown by the naked rachis, were the only ones estimated as sterile.

MATERIAL AND METHODS

During the summer of 1915 the writer had the opportunity of making a detailed study of the occurrence of sterile spikelets in a large number of varieties of wheat under test by the Department of Agronomy at the Delaware Agricultural Experiment Station. These varieties and strains of wheat, 188 in number, had been sown the previous autumn by two methods: First, by a grain drill as under ordinary field conditions, at the rate of 7 pecks per acre; second, by the centgener or hill method, leaving the individual plants 6 inches apart each way. By the former method the plants were very close in the rows, which were 8 inches apart. This gave an opportunity to determine to what degree the closeness of the plants or rate of seeding influenced the frequency of sterile spikelets.

The data for each variety were secured in the following manner: The total number of fertile and sterile spikelets were counted on 25 representative spikes of each variety. The means of the fertile spikelets and the sterile spikelets were taken separately and the percentage of sterile spikelets was determined for each variety of wheat. Where the varieties were planted in hills 6 inches apart each way, five plants of five culms each constituted the 25 spikes, the spikelets of which were counted. In this manner the actual number of sterile spikelets and the percentage of the total number of spikelets were determined for the 188 varieties and strains under the two methods of planting.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dk

Vol. VI, No. 6
May 8, 1916
Del.—2

EFFECT OF RATE OF SEEDING ON STERILITY OF SPIKELETS

It was found (see Table I) that the actual number of sterile spikelets per spike (average of 25 spikes) ranged from 1.84, the lowest, to 5.52, the highest, for varieties in drills; and in hills, from 0.28 sterile spikelets, the lowest, to 3.76, the highest. The percentage of sterile spikelets per average spike in drill rows ranged from 11.5 per cent, the lowest, to 36 per cent, the highest. In hills the percentage of sterile spikelets among the varieties ranged from 1.5 per cent to 23.5 per cent. The mean number of sterile spikelets for all varieties in drill rows was 3.47; in hills, 1.73. The mean percentage of sterile spikelets for all varieties in drills was 21.8 per cent; in hills, 10 per cent.

The data indicate that the spacing of the wheat plants has a direct bearing on the number of sterile spikelets. Wheat planted in hills has more space in which to develop and invariably sends up a greater number of tillers than wheat sown in drills. It has also been observed that the period of maturation is prolonged where the wheat plant has more space. Under these conditions the vegetative activity of the plant is more pronounced, as shown by an increased number of culms, broader leaves, and heavier straw.

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*

Variety.	Bearded or smooth.	Total number of spikelets.		Number of sterile spikelets.		Percentage of sterile spikelets.	
		Drill.	Hill.	Drill.	Hill.	Drill.	Hill.
Acme	B	15.36	15.80	3.56	3.72	23.69	23.54
Acme Bred (Maryland)	B	14.92	15.08	2.96	2.84	19.84	18.83
Acme Improved (Maryland)	B	13.12	15.68	3.04	2.80	23.17	17.83
Ahrens (Indiana)	S	16.92	17.92	2.36	1.56	13.94	8.70
American Banner	S	16.04	16.92	2.76	2.20	17.20	13.53
American Bronze	S	17.88	19.96	3.72	2.88	20.80	14.48
Babcock (Michigan 07664)	B	14.64	14.72	3.60	2.96	24.59	20.10
Bearded Purple Straw	B	15.48	15.04	3.44	2.12	22.22	14.09
Bearded Winter (Michigan 9850)	B	16.28	16.28	3.76	2.56	23.09	15.72
Bearded Winter Fife	B	18.28	17.48	3.80	2.48	20.79	14.18
Beechwood Hybrid	S	15.50	16.16	2.48	1.68	16.00	10.39
Beloglina	B	15.78	13.52	2.84	1.88	17.99	13.90
Berkeley	B	16.12	16.20	3.76	2.12	23.32	13.09
Berkeley Awnless	S	14.12	17.08	2.00	2.32	14.16	13.58
Blue Stem	S	15.44	15.44	2.84	1.24	18.38	8.03
Broughton	S	15.20	15.28	2.68	1.64	17.63	10.73
Buda Dawson (Michigan 310717)	B	14.36	14.80	3.72	1.80	25.90	12.16
Buda Pest	B	15.48	14.48	3.04	1.48	19.63	10.22
Canadian Hybrid	S	17.40	15.80	4.12	1.20	23.67	7.59
Century	S	15.84	16.24	3.00	1.12	18.93	6.89
China	S	17.04	15.68	3.48	2.08	20.42	13.26
Clawsons Longberry	S	17.64	18.24	3.40	2.00	19.27	10.96
Cooks Brookmont	B	13.36	15.16	3.24	2.00	24.25	13.19
Councilman	S	15.32	15.76	2.64	1.36	17.23	8.26
Craigs Favorite	S	15.48	17.44	3.56	1.76	22.99	10.09
Currells Prolific	S	15.52	16.32	3.48	1.12	22.39	6.86
Crimean	S	14.64	17.28	3.48	2.04	23.77	11.80

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

Variety.	Bearded or smooth.	Total number of spikelets.		Number of sterile spikelets.		Percentage of sterile spikelets.	
		Drill.	Hill.	Drill.	Hill.	Drill.	Hill.
Dawsons Golden Chaff	S	16.36	17.32	3.00	2.56	18.33	14.77
Defiance	B	13.60	13.76	3.24	1.60	23.82	11.62
Diamond Grit	B	18.76	18.76	5.28	3.52	28.14	18.76
Dietz	B	15.28	15.44	3.24	1.68	21.20	10.87
Dietz Longberry	B	15.04	16.55	2.86	2.16	19.01	13.19
Doub	B	15.00	13.52	3.12	1.64	20.80	12.13
Dunlap	B	15.24	15.00	3.92	1.84	25.72	12.26
Early Harvest	S	15.60	15.08	2.60	.84	16.66	5.57
Early Red Chief	S	16.24	17.56	2.64	.40	16.25	2.27
Early Red Clawson	S	16.76	16.92	3.56	1.16	21.24	6.85
Early Windsor	S	16.56	17.80	3.48	1.28	21.02	7.19
Eclipse	B	17.24	19.00	3.92	2.40	22.73	12.62
Egyptian Amber	B	17.64	17.08	4.72	2.80	26.75	11.63
Enterprise	S	15.36	16.44	3.40	1.44	22.13	8.75
European Century	B	16.76	18.48	3.32	2.08	19.80	11.25
Farmers Trust	B	18.08	17.40	4.48	2.24	24.77	12.87
Fish Head	B	15.80	18.24	3.00	2.32	18.98	12.71
Fulcaster	B	15.56	15.28	2.96	2.12	19.02	13.87
Four Row Fultz	S	16.56	17.76	2.40	.48	14.49	2.76
Jersey Fultz	S	14.84	15.64	2.72	.60	18.32	3.84
Fultz	S	16.32	17.60	2.36	.56	14.46	3.18
Fultz Mediterranean	S	16.40	16.92	1.96	.28	11.95	1.59
Genessee Giant	B	18.04	19.44	4.20	1.28	23.28	6.58
Giant Square Head	B	17.40	18.36	4.28	.64	24.59	3.48
Goens	B	15.36	15.00	3.24	1.04	21.09	6.92
Goens Awnless	S	15.44	15.32	2.48	.76	16.06	4.96
Gill	S	15.44	15.12	2.52	.36	16.32	2.38
Glace	S	19.00	19.80	4.68	2.08	24.63	10.50
Gold Coin	S	17.24	17.36	3.60	1.44	20.88	8.29
Golden Bronze	S	16.24	18.32	3.20	2.04	19.70	11.13
Greening (Michigan 126)	S	16.04	17.92	3.52	1.80	21.94	10.04
Gypsy	B	17.76	18.84	4.16	2.24	23.42	11.88
Hedges Prolific	S	15.32	16.80	2.80	.44	18.27	2.16
Hercules	B	15.16	17.08	3.20	1.88	21.10	11.00
Harvest King	S	15.72	16.20	2.64	.60	16.79	3.70
Hickman	S	15.45	15.68	2.44	1.48	15.84	9.43
Hungarian (Michigan 913802)	B	14.92	17.96	3.92	1.80	26.27	10.02
Hybrid Sel. 13	B	18.00	22.08	3.84	2.56	21.33	11.59
Hyde Michigan 6	S	16.84	19.88	3.24	2.12	19.23	10.66
Imperial Amber	B	15.92	18.68	4.04	2.56	25.37	13.70
International 6 (Michigan 61)	S	15.63	18.00	3.20	1.88	20.47	10.44
Jones Early Red Chaff	S	15.80	17.76	3.28	.92	20.75	5.18
Jones Longberry	S	18.32	17.88	4.28	.48	23.36	2.68
Jones Mammoth Amber	B	18.96	22.68	5.00	2.36	26.37	10.40
Jones Paris Prize	S	16.88	18.12	3.00	1.48	17.77	8.22
Jones Winter Fife	S	19.60	20.20	3.08	1.04	15.71	5.14
Kansas Mortgage Lifter	B	14.64	16.20	2.60	1.44	17.76	8.88
K. B. 2	S	18.44	20.12	3.84	1.68	20.82	8.34
Kharkov	B	13.96	15.76	3.16	1.16	22.63	7.36
Klondike	S	16.84	17.92	3.16	1.32	18.76	7.42
Lancaster-Fulcaster	B	14.32	15.24	3.16	1.44	22.06	9.44
Lancaster Red	B	15.84	16.36	4.12	1.52	26.01	9.29
Lebanon	B	14.88	15.84	3.52	2.16	23.65	13.63
Mammoth Red	B	15.80	16.84	2.88	2.00	18.22	11.87
Martins Amber	S	18.24	21.00	4.08	2.32	22.36	11.04
Malakoff	B	14.24	14.96	2.48	1.64	17.41	10.96
Massey	S	16.88	20.04	2.44	2.24	14.45	11.12

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

Variety.	Bearded or smooth.	Total number of spikelets.		Number of sterile spikelets.		Percentage of sterile spikelets.	
		Drill.	Hill.	Drill.	Hill.	Drill.	Hill.
Mealy	S	17.72	20.00	2.84	2.16	16.02	10.80
Mediterranean	B	15.20	16.00	3.68	1.96	24.12	12.25
Michigan Amber	S	15.92	17.52	3.00	1.64	18.84	9.36
Millers Pride	B	14.20	16.84	2.64	2.20	18.59	13.06
Miracle	B	15.72	16.84	4.40	1.80	27.98	10.68
Missing Link	B	17.76	19.84	4.64	2.48	26.12	12.50
Morse	S	14.48	16.08	2.24	.56	15.47	3.48
New Amber Longberry	B	18.80	20.16	4.84	1.40	25.73	6.99
New Soules	S	17.16	17.80	3.58	1.24	20.86	6.96
Nigger	B	12.80	14.40	2.68	1.56	20.93	10.83
Nixon	S	15.96	15.60	3.50	.84	21.92	5.38
Ohio 5507	S	17.28	17.92	3.72	1.04	21.52	5.80
Ontario Wonder	S	17.80	20.32	4.20	1.76	23.59	8.66
Orange	S	15.08	15.96	2.72	1.68	18.03	10.53
Pesterboden	B	15.16	15.96	2.80	1.80	18.46	11.90
Perfection	S	14.92	16.44	2.92	.60	19.57	3.64
Plymouth Rock	S	16.72	18.88	3.52	2.00	21.05	10.59
Poole	S	15.64	18.20	2.72	2.28	17.39	12.52
Pride of Genessee	B	18.76	21.48	5.32	3.76	28.35	17.50
Prosperity	S	16.84	19.56	3.24	1.60	19.23	8.17
Purple Straw	B	15.28	20.44	2.72	2.32	17.80	11.35
Red Cross	S	16.60	20.32	3.44	1.24	20.72	6.10
Red Hussar	B	12.76	18.12	2.88	.72	22.57	3.97
Red Rock	B	13.32	15.68	3.36	1.92	25.22	12.24
Red Wave	S	18.16	19.52	4.24	1.56	23.34	7.83
Reiti	B	17.20	21.60	4.44	2.20	25.81	10.18
Reliable	B	15.88	18.68	3.84	2.24	24.18	11.99
Rochester Red	S	15.96	18.80	3.96	1.68	24.81	8.93
Rocky Mountain	B	15.00	15.68	3.96	1.92	26.40	12.24
Royal Red Clawson	S	14.96	16.52	3.24	.48	21.65	2.90
Rudy	B	12.52	14.20	2.72	1.04	20.92	7.32
Rudy Hard	B	13.84	16.12	2.92	1.32	21.09	8.18
Ruperts Giant	S	17.24	19.80	4.40	1.60	25.51	8.08
Rural New Yorker	S	16.92	19.56	3.72	1.44	21.98	7.36
Russian Amber	B	15.84	19.52	4.80	2.76	30.30	14.13
Shepherds Perfection	B	16.64	20.56	4.56	2.80	27.40	13.61
Silver Sheath	B	13.52	17.52	3.68	2.40	27.14	13.69
Silver Wave	B	17.32	19.48	4.76	2.88	27.48	14.79
Smiths Rustproof	S	16.72	20.68	4.28	2.72	25.59	13.15
Soumans Champion	B	16.80	19.28	3.76	1.88	22.38	9.74
Spayde	B	16.52	19.76	4.16	1.56	25.18	7.89
St. Louis Grand Prize	S	18.00	20.40	3.44	1.24	19.11	6.07
Stone	B	13.00	16.96	3.84	2.32	29.53	13.68
Swamp	B	14.88	17.88	3.68	.84	24.73	4.69
Theiss	B	13.28	17.52	3.84	1.60	28.91	9.70
Turkey Red	B	14.44	16.60	2.76	1.72	19.11	10.37
Turkish Amber	B	15.48	16.88	3.28	1.24	21.18	7.34
Velvet Chaff	B	16.80	18.04	4.04	1.88	24.04	10.42
Valley		16.68	17.76	4.60	2.32	27.57	13.06
Wayside Wonder	B	14.32	17.08	3.92	1.68	27.37	9.83
Whedling	S	13.80	15.60	2.40	.92	17.39	5.89
White Eldorado	S	15.96	18.36	3.48	1.24	21.80	6.75
Wyandotte Red	S	14.64	16.96	2.80	1.16	19.12	6.82
Tennessee 3608	B	18.12	22.48	5.52	3.12	30.46	13.87
Tennessee 3609	B	17.60	19.68	4.40	2.20	25.00	11.17
Tennessee 3611	B	15.16	20.16	4.32	2.76	28.49	13.69
Tennessee 3614	B	15.28	18.40	3.80	2.40	24.86	13.04

TABLE I.—*Number and percentage of sterile spikelets on 25 spikes of bearded and smooth varieties of wheat in 1915*—Continued

Variety.	Bearded or smooth.	Total number of spikelets.		Number of sterile spikelets.		Percentage of sterile spikelets.	
		Drill.	Hill.	Drill.	Hill.	Drill.	Hill.
Tennessee 3617	B	17.16	20.44	4.68	2.36	27.27	11.54
Tennessee 3277	B	18.36	21.60	5.40	2.64	29.41	12.22
U. S. 2980	B	14.84	17.68	2.92	2.00	19.67	11.70
U. S. 3608	B	17.92	21.24	4.84	2.72	27.06	12.80
U. S. 3609	B	17.12	19.68	4.36	2.20	25.46	11.17
U. S. 3610	S	18.16	21.56	3.52	1.68	19.38	7.79
U. S. 3612	B	17.52	21.36	4.48	2.52	25.57	11.79
U. S. 3613	B	15.00	20.40	3.92	2.56	26.13	12.54
U. S. 3614	B	16.40	19.20	3.40	1.80	20.73	9.36
Abundance	S	16.44	17.76	2.60	2.00	15.81	11.26
Auburn Red	B	16.08	16.88	3.40	2.32	21.15	13.74
Australian Red	B	14.52	15.84	3.24	2.12	22.31	13.38
Banat	B	13.72	15.52	3.48	2.64	25.36	17.01
Bulgarian	B	14.88	16.68	4.16	2.04	27.95	12.23
California Red	S	14.96	15.76	2.88	.32	19.25	2.03
Davidson	S	15.95	17.44	1.84	.80	11.52	4.58
Deitz Amber	B	14.52	15.88	3.80	1.24	26.17	7.80
Deitz Mediterranean	B	14.44	15.64	3.50	1.32	24.23	8.43
Early Pearl	S	13.04	13.92	2.56	.72	19.64	5.17
Early Ripe	S	15.08	16.08	2.80	.68	18.56	4.22
Economy	S	14.48	15.36	2.72	1.16	18.78	7.55
Egyptian	B	14.08	17.20	5.08	1.80	36.07	10.46
Farmers Friend	B	13.00	14.40	2.96	1.68	22.76	11.69
Ghirka Winter	B	15.76	18.56	3.92	2.12	28.88	11.36
Goings	B	15.16	14.80	3.00	1.04	19.78	7.02
Grand Prize	S	17.76	19.72	3.36	2.00	18.91	10.14
Invincible	S	17.92	20.60	4.36	2.32	24.33	11.26
Jones Red Wave	S	18.20	20.21	4.24	1.64	23.29	8.15
Kentucky Bluestem	S	14.84	17.28	3.12	1.40	21.02	8.10
Lancaster	B	14.36	15.16	3.48	1.96	24.23	12.92
Lehigh	B	13.96	17.16	3.52	2.88	25.21	16.78
Petigree Giant	B	18.24	19.40	3.80	1.40	20.83	7.21
Red May	S	14.32	16.84	2.64	.36	18.43	2.13
Reiti	B	14.72	17.88	3.44	1.68	25.14	9.39
Sibleys New Golden	B	14.44	17.84	3.56	1.52	24.65	8.54
Texas Red	B	13.04	17.28	3.80	1.76	29.14	16.18
Treadwell	B	15.24	18.00	4.60	1.72	30.18	9.55
Tuscan Island	B	15.32	16.72	3.92	2.00	25.58	11.96
Ulta	B	13.32	16.04	3.80	1.24	28.52	7.73
Winter Chief	S	15.44	17.56	2.40	.44	15.54	2.50
Winter King	B	14.16	15.04	3.24	1.28	22.88	8.51
Wisconsin 13	B	13.12	16.36	3.76	1.24	28.65	7.58
Leaps Prolific	S	17.06	18.88	2.28	.68	13.36	3.60
Average		15.85	17.13	3.47	1.73	21.88	10.09

Of the 188 varieties and strains of wheat under observation, 108 were beardless and 80 bearded. To determine whether the presence or absence of awns as a morphological character was in any way correlated with the occurrence of sterile spikelets, the varieties were tabulated so as to show the distribution of bearded and of beardless varieties with reference to the percentage of spikelets (see Table II). The data in this case were taken from the varieties sown in drills.

TABLE II.—*Arrangement of bearded and beardless varieties of wheat with reference to the percentage of sterile spikelets*

Percentage of barren spikelets.	Total number of varieties.	Number of beardless varieties.	Number of bearded varieties.	Percentage of each class to total number of—	
				Beardless varieties.	Bearded varieties.
11 to 15	8	8	0	10.0	0
15 to 17	12	12	0	15.0	0
17 to 19	27	19	8	23.7	7.4
19 to 21	32	18	14	22.5	12.9
21 to 23	30	14	16	17.5	14.8
23 to 25	31	7	24	8.7	22.2
25 to 27	25	2	23	2.5	21.2
27 to 29	17	0	17	0	15.7
29 to 31	5	0	5	0	5.5
Total	188	80	108	100.0	100.0

Table II shows that the bearded varieties as a class have a higher percentage of sterile spikelets than the beardless wheats. There are 20 of the 80 varieties of beardless wheat which have more than 15 per cent of sterile spikelets, while not a single variety of bearded wheat has less than 17 per cent of sterile spikelets. Of the 108 bearded varieties 45 have not less than 25 per cent of sterile spikelets. Only two of the 80 beardless varieties have 25 per cent of sterile spikelets. The average percentage of sterile spikelets for all the beardless varieties is 17.8; for the bearded, 24.1; a difference of 6.1 per cent in favor of the beardless varieties. The individual variety having the lowest percentage, 11.5, was beardless, while the variety having the highest percentage of sterile spikelets, 36.7, was bearded. All of the varieties which are mentioned above were sown under like conditions of soil preparation and fertilization and planted at the same time.

EFFECT OF TIME OF SEEDING ON STERILITY

The next step was to determine the effect of time of seeding and of soil treatment on the frequency of sterile spikelets. As it happened, an experiment was already under way on different dates of sowing wheat, including two varieties, one bearded and the other beardless, on both fertilized and unfertilized soil. These plants were in hills 6 inches apart each way. In the manner followed above, the total number of spikelets and that of sterile spikelets per spike were combined, and the average was determined for the two varieties under different dates of planting on both treated and untreated soil (Table III).

TABLE III.—*Effect of date of planting on the number of sterile spikelets in 25 spikes of two varieties of wheat on fertilized and on unfertilized soil*

RED WAVE (BEARDLESS)

Date of planting.	Total number of spikelets.		Number of sterile spikelets.		Percentage of sterile spikelets.	
	Fertilizer.	No fertilizer.	Fertilizer.	No fertilizer.	Fertilizer.	No fertilizer.
Sept. 17	21.4	17.7	2.8	2.1	13.4	12.1
24	20.5	18.4	2.2	1.8	11.1	9.7
Oct. 1	20.0	20.3	2.2	2.2	11.1	10.8
8	21.5	18.8	2.1	1.5	10.1	8.4
15	21.3	19.9	2.2	1.6	10.6	8.4
22	19.7	20.9	1.0	1.2	5.4	5.7
Average	20.7	19.3	2.1	1.7	10.3	9.3

MIRACLE (BEARDED)

Sept. 17	16.7	15.0	2.3	1.5	13.8	10.4
24	16.2	14.9	2.6	1.2	16.4	8.5
Oct. 1	18.9	15.5	2.6	1.8	14.1	11.5
8	16.0	15.4	3.1	1.8	19.7	12.1
15	15.7	16.8	1.6	1.8	10.1	10.9
22	16.1	15.4	.9	.4	5.7	2.8
Average	16.6	15.5	2.2	1.4	13.3	9.4

Table III shows that the number of sterile spikelets per spike varies considerably from the earliest seeding, September 17, to the latest, October 22, but in no regular manner. The latest seeding in every case shows the smallest number of sterile spikelets. This holds true for both varieties and under both soil conditions. If the average is taken of the number of sterile spikelets under the six different dates of seeding, it is found that there are more sterile spikelets where fertilizer was used than where no application was made. This also holds true for both varieties. Expressed as a percentage, the average of sterile spikelets for the different rates of seeding with the beardless variety is 10.3 per cent where fertilizer was used and 9.3 per cent on untreated soil. That of the bearded variety was 13.3 per cent of sterile spikelets as an average for the different dates of seeding on treated soil and 9.4 per cent on the untreated. It will be noted that the latest seeding of each variety has as many spikelets as the earliest, and that there are more than twice as many sterile spikelets in the latter than in the former. This may be partially accounted for by the fact that the later plantings did not have a full stand of plants, thus giving the individual wheat plant more space. This explanation is in accord with results obtained under the different methods of seeding (see Table I)—that is, that fewer sterile spikelets were found in the thinner plantings.

The tillering in the early plantings was nearly 100 per cent greater than in the later plantings. The tillering for each variety on fertilized soil for a given date was 50 per cent greater than where no fertilizer was used. The general effect of the date of seeding seems to indicate a tendency toward a smaller percentage of sterile spikelets in the later seedings. The relation of the number of sterile spikelets to yield does not seem to affect the yield seriously, since the fertilized wheats produced two or three times as much grain per spike as the unfertilized. The difference in yield per spike seems to be due largely to quality (size) of kernel.

TABLE IV.—*Relation of the effect of different fertilizers and combinations of fertilizers to the occurrence of sterile spikelets*

Treatment.	Dawsons Golden Chaff (smooth).			Lehigh (bearded).		
	Total number of spikelets.[1]	Number of sterile spikelets.[1]	Percentage of sterile spikelets.	Total number of spikelets.[1]	Number of sterile spikelets.[1]	Percentage of sterile spikelets.
Nitrogen, phosphorus, and potassium	16.8	1.36	8.0	17.9	1.32	7.3
Nitrogen and phosphorus	18.2	1.68	9.2	18.2	2.08	11.4
Phosphorus and potassium	18.2	1.92	10.2	17.2	1.80	10.4
Nitrogen and potassium	18.2	1.08	5.9	17.3	.92	5.2
None	17.0	1.05	6.1	16.7	1.01	6.0
Nitrogen	16.9	.92	5.4	18.0	1.36	7.5
Phosphorus	15.9	1.56	9.7	15.0	1.40	9.2
Potassium	17.0	1.32	7.7	16.8	1.24	7.3

[1] Average of 25 spikes.

EFFECT OF FERTILIZERS ON STERILITY

The effect of different elements of plant food, singly and in combination, on the number of sterile spikelets is seen in Table IV. The wheat was planted by the centgener method, the individual plants being 6 inches apart each way. On each of the plots sufficient fertilizer of each mineral ingredient was supplied to produce a 50-bushel crop of wheat, provided that it were all used. The nitrogen was applied for a 25-bushel crop, it being assumed that the soil carried a fair reserve of this element. The nitrogen was applied in equal parts by weight of nitrate of soda and dried blood; the phosphoric acid was carried as acid phosphate and the potash as muriate of potash. It will be noted that where the fertilizers were applied singly nitrogen gave the lowest percentage—6.4—of sterile spikelets as an average for the two varieties. Potash came next with 7.5 per cent, and phosphoric acid stood highest, with 9.4 per cent of sterile spikelets. Where two elements were used in combination, phosphoric acid and potash led, with an average of

10.4 per cent for the two varieties; phosphoric acid and nitrogen combined gave 10.3 per cent of sterile spikelets, while nitrogen and potash gave 5.4 per cent. Since phosphoric acid gave the highest percentage of sterile spikelets when used alone, it would seem that this element of plant food is largely responsible for the sterile spikelets, as in every combination in which it is used the number of sterile spikelets is greater than where nitrogen and potash are used singly or in combination. The untreated plot gave 6 per cent of sterile spikelets, the lowest for the series except where nitrogen and potash were used in combination, which gave 5.5 per cent. The complete fertilizer gave an average of 7.6 per cent of sterile spikelets. From these data it would seem that there is a tendency for phosphoric acid to produce a larger percentage of sterile spikelets than either potash or nitrogen. However, the fairly high percentage of sterile spikelets in the case of the wheat treated with phosphoric acid did not affect the yield per plant or spike. Under this treatment the yield and quality of the grain surpassed that under either nitrogen or potash.

CORRELATIONS

In order to determine what relation might exist between the total number of spikelets per spike and the number of sterile spikelets, the readings constituting the averages for the 25 spikes of each variety were arranged in correlation tables. The beardless varieties form one table and the bearded the other. Thus, the readings were the average of each variety and the array or distribution in the table was made up of varieties. The data were secured from the plants in hills. Since the number of spikelets per spike in a large measure determines the length of spike, the relation found will be closely associated with the length of the spike. In Table V, which includes the beardless varieties, the coefficient of correlation between the number of sterile spikelets and the total number of spikelets is 0.543 ± 0.054. The bearded varieties show a correlation which is expressed as $r = 0.598 \pm 0.041$. It appears that the number of sterile spikelets per variety bears a direct positive correlation to the total number of spikelets or the length of head. The varieties with the shorter spikes have decidedly fewer sterile spikelets. The relation between the number of spikelets and the length of spike may not be close, inasmuch as there may be more or less range among varieties as to the condensation or closeness of the spikelets on the spike. However, the long spikes are made up of a relatively larger number of spikelets than the short ones, and the actual percentage of sterile spikelets may be smaller in the long spikes, as will be pointed out later.

TABLE V.—*Correlation between the number of sterile spikelets and the total number of spikelets in beardless and bearded varieties of wheat*

BEARDLESS VARIETIES[1]

Number of sterile spikelets.	12 to 13.	13 to 14.	14 to 15.	15 to 16.	16 to 17.	17 to 18.	18 to 19.	19 to 20.	Total.
1 to 2	.....	.....	.....	1	1	1	.....	.....	3
2 to 3	.....	2	8	14	7	1	.....	.....	32
3 to 4	.....	.....	2	10	12	7	3	1	35
4 to 5	.....	.....	.....	.....	1	4	3	1	9
Total	.....	2	10	25	21	13	6	2	79

BEARDED VARIETIES[2]

1 to 2	.....	.....	.....	.....	.....	.....	.....	.....	0
2 to 3	4	2	6	6	.....	.....	.....	.....	18
3 to 4	.....	16	15	18	6	.....	3	.....	58
4 to 5	.....	.....	1	7	4	11	4	.....	27
5 to 6	.....	.....	1	.....	.....	.....	5	.....	6
Total	4	18	23	31	10	11	12	.....	109

[1] $r = 0.543 \pm 0.054$. [2] $r = 0.598 \pm 0.041$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND OTHER CHARACTERS OF THE WHEAT PLANT

For the purpose of studying the relationship between the percentage of sterile spikelets per plant and other characters, 300 plants of the variety Velvet Chaff were pulled, dried, and later carefully measured. The plants had been grown by the centgener method, 6 inches apart each way. The percentage of sterile spikelets was used rather than the actual number, for the reason that the length of spikes, which determines the number of spikelets, varies so greatly. The measurements of length were taken in centimeters and those of weight in milligrams. Biometrical data were secured for the statistical relationship between the percentage of sterile spikelets per plant and (1) the number of culms per plant; (2) the yield of grain per plant; (3) the yield of grain per spike; (4) the length of the culm; (5) the length of the spike; (6) the average weight of the kernel; and (7) the number of spikelets. In the above determinations the plant was used as a unit, the value for each character being determined by taking the average of the respective readings.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE NUMBER OF CULMS PER PLANT

An inspection of Table VI shows only a slight degree of correlation between the percentage of sterile spikelets and the number of culms, which is negative. The coefficient of correlation is -0.076 ± 0.039. Evidently there exists no appreciable relationship between the percentage of

sterile spikelets and the number of tillers per plant. The less vigorous plants, indicated by the smaller number of tillers per plant, do not show a higher percentage of sterile spikelets than the more thrifty plants.

TABLE VI.—*Correlation between the percentage of sterile spikelets per plant and the number of tillers per plant in wheat*[1]

Percentage of sterile spikelets per plant.	Number of tillers per plant.													Total.
	1	2	3	4	5	6	7	8	9	10	11	12	13	
0 to 3	1	2	....	1	....	1	....	1	....	....	....	....	....	6
3 to 7	2	2	13	4	7	5	8	2	1	1	1	....	....	46
7 to 11	2	3	4	15	19	16	14	7	2	....	1	1	1	85
11 to 15	....	10	14	20	11	11	14	6	3	4	1	1	....	95
15 to 19	1	2	7	10	11	4	5	2	1	1	2	1	1	48
19 to 23	2	1	4	1	2	1	....	....	....	....	....	....	....	11
23 to 27	....	1	4	2	....	....	....	....	....	....	....	....	....	7
27 to 31	....	1	....	....	....	....	....	....	....	....	....	....	....	1
31 to 35	....	....	....	....	....	....	1	....	....	....	....	....	....	1
Total	8	22	46	53	50	38	42	18	7	6	5	3	2	300

[1] $r = -0.0756 \pm 0.0387$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE YIELD OF GRAIN PER PLANT

Between the percentage of sterile spikelets and the yield of grain per plant (Table VII) the coefficient of correlation is negative, -0.306 ± 0.035. This correlation is fairly high and though expressed negatively indicates that the higher yielding plants have a smaller percentage of sterile spikelets than those of low yield.

TABLE VII.—*Correlation between the percentage of sterile spikelets per plant and the yield of grain per plant in wheat*[1]

Percentage of sterile spikelets per plant.	Yield of grain per plant (in milligrams).													Total.
	0 to 500	500 to 1,000	1,000 to 1,500	1,500 to 2,000	2,000 to 2,500	2,500 to 3,000	3,000 to 3,500	3,500 to 4,000	4,000 to 4,500	4,500 to 5,000	5,000 to 5,500	5,500 to 6,000	6,000 to 6,500	6,500 to 7,000	
0 to 3	....	1	2	2	....	....	....	....	....	....	1	....	....	....	6
3 to 7	1	5	7	6	6	7	2	5	2	3	1	1	....	....	46
7 to 11	2	3	12	15	23	10	10	4	....	2	1	1	1	1	85
11 to 15	6	16	20	16	9	13	5	3	4	1	....	1	....	1	95
15 to 19	7	8	12	4	4	7	2	....	1	2	....	....	....	1	48
19 to 23	5	3	1	1	1	....	....	....	....	....	....	....	....	....	11
23 to 27	2	5	....	....	....	....	....	....	....	....	....	....	....	....	7
27 to 31	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
31 to 35	....	1	....	....	....	....	....	....	....	....	....	....	....	....	1
Total	24	42	54	44	43	37	19	12	7	8	3	3	1	3	300

[1] $r = -0.3057 \pm 0.0353$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE AVERAGE YIELD OF GRAIN PER SPIKE

The average yield of grain per spike (Table VIII) was determined by dividing the total weight of grain per plant by the number of spikes per plant. The coefficient of correlation between this yield and the percentage of sterile spikelets is again negative, -0.589 ± 0.025, which indicates a much closer relationship between the low percentage of sterile spikelets and yield of grain per spike than is shown between the same character and the yield per plant. There is a rather high correlation existing between the percentage of sterile spikelets and the yield of grain per spike.

TABLE VIII.—*Correlation between the percentage of sterile spikelets per plant and the average yield of grain per spike in wheat* [1]

Percentage of sterile spikelets per plant.	Yield of grain per spike (in milligrams).																Total.
	50 to 100	100 to 150	150 to 200	200 to 250	250 to 300	300 to 350	350 to 400	400 to 450	450 to 500	500 to 550	550 to 600	600 to 650	650 to 700	700 to 750	750 to 800	800 to 850	
0 to 3	...	...	....	....	....	1	....	....	1	2	....	...	1	...	1	...	6
3 to 7	...	...	1	1	2	2	3	7	8	7	6	1	1	6	...	1	46
7 to 11	...	...	....	4	9	7	17	12	14	13	6	2	...	1	...	...	85
11 to 15	...	2	6	11	12	17	16	13	8	3	4	2	...	...	...	...	95
15 to 19	2	3	2	11	8	8	9	2	1	1	....	1	...	...	...	...	48
19 to 23	2	1	1	2	3	1	1	....	....	....	....	....	...	...	...	...	11
23 to 27	...	...	2	4	1	....	....	....	....	....	....	....	...	...	...	...	7
27 to 31	...	1	....	....	....	....	....	....	....	....	....	....	...	...	...	...	1
31 to 35	...	1	....	....	....	....	....	....	....	....	....	....	...	...	...	...	1
Total	4	8	12	33	36	36	46	34	32	26	16	6	2	7	1	1	300

[1] $r = -0.5888 \pm 0.0254$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE AVERAGE LENGTH OF CULM PER PLANT

In this case the average length of culm per plant (see Table IX) was found by taking the sum of the lengths of the culms of a plant in centimeters and dividing it by the number of culms. The correlation coefficient is -0.448 ± 0.031. This is a rather high degree of correlation and is expressed as negative, although with reference to the relation of the two characters compared it means that the longer culms tend to form a lower percentage of sterile spikelets. This is what might be expected, since the yield of grain per spike is generally closely associated with the length of spike, and that in turn with the length of culm.

TABLE IX.—*Correlation between the percentage of sterile spiklets per plant and the average length of culm in wheat* [1]

Percentage of sterile spikelets per plant.	Length of culm (in centimeters).												Total.
	60 to 65	65 to 70	70 to 75	75 to 80	80 to 85	85 to 90	90 to 95	95 to 100	100 to 105	105 to 110	110 to 115	115 to 120	
0 to 3	....	....	....	....	....	1	2	3	....	....	....	....	6
3 to 7	....	....	....	....	5	6	7	9	12	5	1	1	46
7 to 11	....	....	....	3	11	13	17	15	19	6	1	....	85
11 to 15	....	1	2	6	16	21	21	15	9	3	1	....	95
15 to 19	....	2	....	7	13	9	8	7	....	2	....	....	48
19 to 23	1	....	1	1	4	2	2	....	....	....	....	....	11
23 to 27	....	1	1	1	1	3	....	....	....	....	....	....	7
27 to 31	....	1	....	....	....	....	....	....	....	....	....	....	1
31 to 35	....	....	....	1	....	....	....	....	....	....	....	....	1
Total	1	5	4	19	50	55	57	49	40	16	3	1	300

[1] $r = -0.4482 \pm 0.0316$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE LENGTH OF SPIKE PER PLANT

The average length of spike per plant was determined by dividing the sum of the lengths of the spikes per plant by the number of spikes. The calculations were expressed in centimeters. The coefficient of correlation between these two characters is -0.451 ± 0.031 (see Table X). Since the longest spikes usually occupy the longest culms, we should expect the same relationship between the length of spike and percentage of sterile spikelets as was found between the latter character and the length of culm (see Table IX). There is a very close relation, the coefficient of correlation with the culm being 0.448 ± 0.031, a difference of 0.003 between the two coefficients.

TABLE X.—*Correlation between the percentage of sterile spikelets per plant and the average length of spike in wheat* [1]

Percentage of sterile spikelets per plant.	Length of spike (in centimeters).																		Total.
	5.4 to 5.8	5.8 to 6.2	6.2 to 6.6	6.6 to 7	7 to 7.4	7.4 to 7.8	7.8 to 8.2	8.2 to 8.6	8.6 to 9	9 to 9.4	9.4 to 9.8	9.8 to 10.2	10.2 to 10.6	10.6 to 11	11 to 11.4	11.4 to 11.8	11.8 to 12.2	12.2 to 12.6	12.6 to 13	
0 to 3	....	....	....	....	....	....	....	1	1	....	....	....	2	2	....	....	....	....	....	6
3 to 7	....	....	....	....	....	....	1	3	5	5	12	8	6	4	....	2	....	....	....	46
7 to 11	....	....	....	....	....	3	4	6	19	14	17	14	5	2	....	....	....	1	....	85
11 to 15	1	....	1	2	3	11	11	12	17	15	10	4	3	1	2	1	....	....	1	95
15 to 19	....	1	....	....	2	3	12	8	4	8	5	2	1	....	1	1	....	....	....	48
19 to 23	....	....	1	1	....	2	4	1	....	1	....	....	....	1	....	....	....	....	....	11
23 to 27	....	....	1	1	3	1	....	....	....	....	1	....	....	....	....	....	....	....	....	7
27 to 31	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	1
31 to 35	....	....	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1
Total	1	1	3	5	8	21	32	31	46	43	45	28	17	10	3	4	0	1	1	300

[1] $r = 0.4515 \pm 0.0310$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS AND THE AVERAGE WEIGHT OF KERNEL

To get the average weight of kernel per plant the total weight of kernels per plant was divided by the number of kernels and the result expressed in milligrams. The coefficient of correlation is -0.421 ± 0.032 (see Table XI). This indicates a decided tendency for the heavier kernels to be associated with a low percentage of sterile spikelets. This is in accord with the relations found to exist between the length of culm and spike and the percentage of sterile spikelets. The more vigorous plants, as indicated by an increased length of culm and spike, generally bear kernels of a larger size. Hence, the correlation between the percentage of sterile spikelets and the weight of kernel—in other words, the quality of the grain—is in the same direction and approximates the other coefficients very closely.

TABLE XI.—*Correlation between the percentage of sterile spikelets per plant and the average weight of the kernel in wheat* [1]

Percentage of sterile spikelets per plant.	Weight of kernel (in milligrams).												Total.
	2 to 4	4 to 6	6 to 8	8 to 10	10 to 12	12 to 14	14 to 16	16 to 18	18 to 20	20 to 22	22 to 24	24 to 26	
0 to 3	....	....	....	....	....	....	1	3	1	....	1	....	6
3 to 7	....	....	....	2	2	8	4	11	7	8	2	2	46
7 to 11	....	....	....	1	6	17	15	26	13	4	1	2	85
11 to 15	....	1	3	6	13	11	29	13	13	5	....	1	95
15 to 19	1	1	1	6	7	15	10	5	1	1	....	....	48
19 to 23	....	....	3	1	3	1	1	2	....	....	....	....	11
23 to 27	....	....	....	....	2	3	2	....	....	....	....	....	7
27 to 31	....	....	1	....	....	....	....	....	....	....	....	....	1
31 to 35	....	....	....	1	....	....	....	....	....	....	....	....	1
Total	1	2	8	17	33	55	62	60	35	18	4	5	300

[1] $r = -0.4209 \pm 0.0320$.

CORRELATION BETWEEN THE PERCENTAGE OF STERILE SPIKELETS PER PLANT AND THE AVERAGE NUMBER OF SPIKELETS PER SPIKE PER PLANT

A relationship is shown below between the percentage of sterile spikelets per plant and the total number of spikelets per plant. The coefficient of correlation is low, -0.152 ± 0.037 (see Table XII). There is only a slight tendency for plants with a low percentage of sterile spikelets to be associated with a large number of spikelets per plant. As the number of spikelets determines to a large extent the length of the spike, it would be supposed that a greater correlation would exist between the number of spikelets and the percentage of sterile spikelets. This may be explained by the fact that the total number of spikelets includes both fertile and sterile spikelets. Also, there may be more or less variation in the condensation of the spikelets which go to make up the spike.

TABLE XII.—*Correlation between the percentage of sterile spikelets per plant and the average number of spikelets per spike per plant in wheat* [1]

Percentage of sterile spikelets per plant.	Number of spikelets per spike.													Total.
	12	13	14	15	16	17	18	19	20	21	22	23	24	
0 to 3	....	....	....	1	....	1	....	3	....	1	....	....	....	6
3 to 7	....	....	....	1	7	5	14	10	9	....	....	....	....	46
7 to 11	....	....	2	4	7	4	26	27	15	....	....	....	....	85
11 to 15	....	....	1	5	20	20	20	18	7	1	3	....	....	95
15 to 19	1	....	1	1	4	12	10	12	6	....	....	....	1	48
19 to 23	....	1	....	3	....	3	1	1	1	1	....	....	....	11
23 to 27	....	1	1	....	1	2	2	....	....	....	....	....	....	7
27 to 31	....	....	....	....	....	1	....	....	....	....	....	....	....	1
31 to 35	....	....	....	....	....	1	....	....	....	....	....	....	....	1
Total	1	2	5	15	39	49	73	71	38	3	3	0	1	300

[1] $r = -0.1524 \pm 0.0375$.

TABLE XIII.—*Variation constants in wheat*

Plant as the unit.	Mean.	Standard deviation.	Coefficient of variation.
Sterile spikelets....per cent..	11.73 ± 0.198	5.105± 0.141	43.51 ±1.406
Number of tillers per plant....	5.193± .091	2.336± .064	44.98 ±1.468
Yield of grain per plant.mgm..	2,048.333±51.125	1,312.821±36.157	64.09 ±2.381
Yield of grain per spike.mgm..	379.833± 5.475	140.599± 3.872	37.02 ±1.151
Length of culm.....cm..	91.417± .366	9.411± .259	10.29 ± .286
Length of spike.....cm..	9.019± .043	1.113± .031	12.34 ± .344
Weight of kernel.....mgm..	15.033± .151	3.881± .107	25.82 ± .756
Number of spikelets per spike.	17.857± .065	1.688± .046	9.335± .257

Characters.	Coefficient of correlation.
Sterile spikelets and number of tillers per plant	−0.075±0.038
Sterile spikelets and yield of grain per plant.....mgm..	− .306± .035
Sterile spikelets and average yield of grain per spike.....mgm..	− .589± .024
Sterile spikelets and average length of culm per plant.....cm..	− .448± .031
Sterile spikelets and average length of spike.....cm..	− .451± .031
Sterile spikelets and weight of kernel per plant.....mgm..	− .421± .032
Sterile spikelets and the average number of spikelets per spike, per plant	− .152± .037

SUMMARY

(1) The number of sterile spikelets per spike in wheat is directly affected by the rate of seeding or the spacing of the plants. The more space allowed each plant the smaller the number of sterile spikelets on each spike.

(2) The bearded varieties of wheat as a class have a higher percentage of sterile spikelets than the beardless varieties. Of the 188 varieties

examined the smallest number of sterile spikelets was found on a beardless variety and the largest number on a bearded variety.

(3) Early seeding seems to increase the percentage of sterile spikelets on each spike. Wheat seeded very late had the smallest percentage of sterile spikelets.

(4) The application of nitrogen alone as a fertilizer produced the lowest percentage of sterile spikelets. Phosphoric acid singly gave the highest percentage of sterile spikelets, while potash was intermediate as to the percentage of sterile spikelets. Where two elements of fertilizers were combined, phosphoric acid and potash gave the highest percentage of sterile spikelets, with nitrogen and phosphoric acid next and nitrogen and potash last. In every instance the check or untreated plots gave a lower percentage of sterile spikelets than those treated with a complete fertilizer.

(5) There is a distinct correlation between the length of spike as expressed by the number of spikelets and the number of sterile spikelets. As the number of spikelets per spike increases (in other words, the length of spike), the number of sterile spikelets becomes greater. That is, varieties with the shorter spikes tend toward a smaller number of sterile spikelets than the varieties with the longer spikes. However, the percentage of sterile spikelets per spike may be greater among the varieties with the shorter spikes, as was shown to be the case where spikes of varying lengths within a single variety were examined.

(6) There is only a very slight correlation between the percentage of sterile spikelets and the number of tillers to each plant.

(7) The yield of grain per plant is correlated to a fair degree with a low percentage of sterile spikelets.

(8) The weight of the kernel or quality of grain is correlated to a considerable degree with a low percentage of sterile spikelets.

(9) The yield of grain per spike, the length of spike, and the length of culm are strongly correlated with a low percentage of sterile spikelets.

(10) There is a slight correlation between the average number of spikelets per spike and a low percentage of sterile spikelets.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
15 CENTS PER COPY
SUBSCRIPTION PRICE, $3.00 PER YEAR

PLATE XXVII

Comparison of the number of sterile spikelets on bearded and beardless varieties of wheat:

On the left two heads of a bearded variety of wheat showing a large number of sterile spikelets. On the right two heads of a beardless variety showing comparatively few sterile spikelets. Both varieties were grown the same year under like conditions of soil and treatment.

Vol. VI MAY 15, 1916 No. 7

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., MAY 15, 1916 NO. 7

EFFECT OF COLD-STORAGE TEMPERATURES UPON THE PUPÆ OF THE MEDITERRANEAN FRUIT FLY[1]

By E. A. BACK, *Entomologist*, and C. E. PEMBERTON, *Scientific Assistant, Mediterranean and other Fruit-Fly Investigations, Bureau of Entomology*

INTRODUCTION

The use to which cold-storage temperatures may be put as an aid in offsetting the disastrous results of attack by the Mediterranean fruit fly, *Ceratitis capitata* Wied., has already been made the subject of discussion by the writers.[2] In their paper, however, data on the effect of various ranges of temperatures used in commercial cold-storage plants upon the eggs and larval instars only are given. So far as the writers have been able to determine, fruits of almost any variety commonly held in storage are held at temperatures varying from 32° to 45° F., with preference shown to a range of 32° to 36°. The effect upon over 26,000 eggs and 60,000 larvæ of different temperatures, including 32°, 32° to 33°, 33° to 34°, 34° to 36°, 36°, 36° to 40°, 38° to 40°, and 40° to 45°, indicate that no eggs or larvæ survive refrigeration for seven weeks at 40° to 45°, for three weeks at 33° to 40°, or for two weeks at 32° to 33°.

While the greatest danger in the spread of this pest from one country to another lies in the transportation of the larvæ within fruits, there are certain data on record which prove that this pest may be carried long distances in the pupal stage and arrive at its destination in a condition to produce infestation. A fruit-fly pupa (species unknown) was found at Auckland, New Zealand, in soil about the roots of plants imported from Australia.[3] In 1914, Sasscer[4] records the discovery in Washington, D. C., of living pupæ of the papaya fruit fly (*Toxotrypana curvicauda* Gerst.) in a package containing an unknown vine from Mexico. In

[1] The writers wish to acknowledge the assistance given them by Mr. H. F. Willard in obtaining the data recorded in this and in their previous paper. To obtain these data has necessitated much prolonged tedious work extending over three years. In securing the data during 1915, Mr. Willard has not only greatly assisted, but on several occasions during the absence of the writers has been entirely responsible not only for the completion of experiments already started, but for the starting of others.

[2] Back, E. A., and Pemberton, C. E. Effect of cold-storage temperatures upon the Mediterranean fruit fly. *In* Jour. Agr. Research, v. 5, no. 15, p. 657–666. 1916.

[3] Kirk, T.W. Fruit flies. New Zeal. Dept. Agr. Div. Biol. Bul. 22, p. 9. 1909.

[4] Sasscer, E. R. Important insect pests collected on imported nursery stock in 1914. *In* Jour. Econ. Ent., v. 8, no. 2, p. 268–270. 1915.

another instance the same investigator records finding a living adult of the olive fruit fly (*Dacus oleae* Rossi) and a dead adult of another species of fruit fly, apparently *Dacus semispharens* Becker. Both of these species were in a small package containing olive seed from Cape Town, South Africa, after having been en route 28 days. Sasscer states that according to Silvestri it requires from 47 to 49 days in Italy for the pupæ of the olive fruit fly to yield adults; hence, it is possible for this ruinous pest to enter the United States through the eastern ports as pupæ and reach the olive-growing sections of California before adults have emerged.

Such facts as these indicate that the Mediterranean fruit fly may be similarly transported, and emphasize the desirability of recorded data on the effect of cold-storage temperatures upon the pupal stages. Aside from the practical application in the future to quarantines regulating the shipment of fruits, the results given below throw considerable light on conditions governing the distribution of the pest, and help explain the varying severity of its ravages in countries having both semitropical and temperate fruit-growing regions.

HISTORICAL REVIEW

Practically nothing has been published on the effect of cold-storage temperatures upon the pupæ of *Ceratitis capitata*. In 1908 Lounsbury [1] in South Africa reports that in removing fruit infested with *C. capitata* from refrigeration at 38° to 40° F. at the end of 21 and 27 days he found in each instance a single pupa, but that both proved to be dead. The experiments of the writers have demonstrated that these two pupæ were produced by larvæ which formed their puparia before the fruit was placed in storage, as larvæ do not form puparia at temperatures lower than 45° to 48° F.

In 1914 Newman,[2] in Western Australia, placed one box containing 50 newly formed puparia in each of four rooms held, respectively, at 32°, 36°, 45°, and 55° F. At the end of 34 days of refrigeration 25 pupæ were taken from each box held at 32° and 36°, and at the end of 70 days of refrigeration the remaining pupæ held at 32° and 36° and all held at 45° and 55° were removed to the laboratory. None of the pupæ removed yielded adults.

EXPERIMENTAL WORK

Nearly all the experimental work with temperatures lower than 45° F. was carried on in a thoroughly modern three-story cold-storage plant. The temperatures of the rooms in this plant were held quite definitely within certain fixed ranges by hourly inspections made by the storage employees. One experiment was carried on in a second plant where, as indicated in the text, the temperature was subject to considerable fluc-

[1] Lounsbury, C. P. Report of the Government Entomologist, Cape of Good Hope, 1907, p. 56. 1908.

[2] Newman, L. J. Annual report of the officer in charge of the insectary for the year ended June 30, 1914. *In* Ann. Rpt. Dept. Agr. West. Aust. 1914, p. 61. 1915.

tuation. The temperatures 49° to 51°, 52° to 56°, and 54° to 57° were not obtainable in the Honolulu cold-storage plants, hence in experiments at these temperatures ordinary refrigerators were used, as indicated. Usually pupæ of all ages from 1 to 9 or 10 days were obtained for each experiment, in order that varying effects upon pupæ in different stages of development might be noted. The pupæ were sifted from sand beneath host fruits and placed in storage either in bulk of several thousand in large jars or, as was more usual, in smaller lots of from one to several hundreds in vials about 1 inch in diameter and stoppered with cotton. Pupæ were not placed in or on damp sand or soil, as early experimental work indicated no advantage from this treatment when pupæ are subjected to cold-storage temperatures. The humidity of the storage rooms varied between 80° and 91°. After refrigeration the pupæ were removed to the laboratory, where they were daily observed for emergence records.

The term "pupa" is used to designate that period in the life history between the formation of the puparium by the larva and the emergence of the adult.

TEMPERATURE, 32° F.—Of the 13,900 pupæ of all ages subjected to refrigeration at a temperature varying less than half a degree either above or below 32° F. during the experiment, none survived more than 10 days. In Table I are recorded the results of observations on pupæ refrigerated from 2 to 10 days.

TABLE I.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration at 32° F. for from 2 to 10 days*

Age of pupæ on entering storage	Number of pupæ yielding adults after removal to normal temperature after refrigeration for—								
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.
1 day	15	6	3	0	1	0	0	0	0
2 days	20	28	20	17	15	4	0	0	0
3 days	32	21	22	19	4	4	3	1	0
4 days	18	17	11	9	1	0	0	0	0
5 days	28	18	3	1	1	1	0	0	0
6 days	29	27	16	6	1	0	0	0	0
7 days	48	33	13	5	2	4	0	0	0
8 days	52	30	17	7	2	0	0	0	0
9 days	51	33	21	12	4	0	0	0	0

Each lot removed after from 2 to 8 days of refrigeration contained 100 pupæ; hence, the number of pupæ yielding adults represents also the percentage of survival. Very few pupæ survived refrigeration at this temperature for longer than one week. Thus only 3 three-day-old pupæ out of 900 pupæ of all ages survived refrigeration for 8 days, and only 1 three-day-old pupa survived refrigeration for 9 days. While the data in Table I do not show it, the one surviving 9 days of refrigeration was one out of 300 of like age, and one out of 1,900 of all ages. Not one of 4,500 pupæ refrigerated for 10 days survived.

TEMPERATURES OF FROM 33° TO 34° F.—Only 3 out of 207 pupæ held at 33° to 34° F. for 4 days and at 43° to 45° F. for 8 additional days yielded adults.

TEMPERATURES OF FROM 33° to 36° F., AVERAGING 34°.—A total of over 27,097 pupæ were used in experiments to determine the effect of a temperature averaging about 34° F. but varying between 33° and 36° F. Only 1 seven-day-old pupa out of 228 of like age or 1,239 of all ages refrigerated for 16 days yielded an adult; 1,228, 1,164, 1,694, and 1,931 refrigerated for 18, 20, 22, and 25 days were dead on removal from storage. Only 3 out of 272 seven-day-old pupæ, or 1,472 pupæ of all ages, produced adults after refrigeration for 15 days, while only 8 out of 210 eight-day-old pupæ and 3 out of 220 seven-day-old pupæ, or but 11 out of 1,630 pupæ of all ages from one to eight days old when placed in storage, produced adults after refrigeration for 14 days. After refrigeration for 12 days, 12 eight-day-old pupæ, 11 seven-day-old pupæ, 2 six-day-old pupæ, and 8 one-day-old pupæ out of a total of 1,580 pupæ of all ages produced adults. From 1 to 30 adults emerged from lots of all ages of pupæ, totaling 1,519 forms, except from 126 five-day-old pupæ, after refrigeration for 11 days, but from 1 to 3 adults emerged from all lots yielding adults, except from the seven-day-old pupæ, which yielded 30 adults from a total of 265 pupæ.

Refrigeration of 1,685 pupæ of all ages for 9 days did not prove totally fatal to any age. Thus 85 out of 340 eight-day-old pupæ, and 88 out of 390 seven-day-old pupæ produced adults as compared with 3 four-day-old pupæ, 7 three-day-old pupæ, and 2 one-day-old pupæ out of a total of 475 pupæ.

Some adults emerged from lots of pupæ representing all ages on removal from storage after 2, 3, 4, 5, 6, 7, and 8 days of refrigeration. On these days an average of about 1,479 pupæ were removed from storage. The number of pupæ surviving is indicated by the data in Table II.

TABLE II.—*Effect upon pupæ of the Mediterranean fruit fly of refrigeration for from 1 to 8 days at 33° to 36° F.*

Age of pupæ on entering storage.	Number of pupæ yielding adults after removal to normal temperature after refrigeration for—						
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.
1 day	87	59	56	59	16	15	9
2 days	41	12	25	6	8	2	6
3 days	49	76	44	18	12	14	6
4 days	27	47	26	11	4	5	3
5 days	12	21	14	10	10	1	4
6 days	10	15	17	10	5	8	2
7 days	190	405	228	221	188	229	98
8 days	129	153	434	146	216	150	91

The data in Table II are introduced to prove that refrigeration for from 1 to 8 days at this temperature is not fatal, and can not be depended

upon to kill all pupæ. While an average of about 1,479 pupæ were removed each day, the number of pupæ of each age is not known; hence no conclusion can be drawn regarding the relative effect of this refrigeration upon pupæ of different ages. The data given above for 9, 11, 12, 14, 15, and 16 days of refrigeration seem to indicate that the older pupæ withstand the effects of cold for a relatively longer period.

In a second experiment 50 out of 200 pupæ of all ages yielded adults after 4 days' refrigeration, and but 15 out of 207 pupæ held at 33° to 34° F. for 4 days and then at 43° to 45° F. for 3 additional days.

TEMPERATURES OF FROM 28° TO 40° F., AVERAGING 36°.—A total of 8,500 pupæ were placed in a cold-storage room the temperature of which was subject to far greater changes than are usual in commercial plants. While the temperature averaged about 36° F. a large portion of the time, for short periods during the night it dropped to freezing or even 28°, and during the heat of the day when supplies were being removed frequently rose to 38° to 40°. As each lot of the various ages from ½ to 9 days removed consisted of 100 pupæ, the numbers of pupæ yielding adults after the various numbers of days of refrigeration represent the percentages of survival. In Table III are recorded the effects of from 1 to 24 days of refrigeration on 6,800 pupæ.

TABLE III.—*Effect upon pupæ of refrigeration at temperatures varying between 28° and 40° F., but averaging about 36° F.*

Age of pupæ on entering storage.	Number of pupæ yielding adults on removal to normal temperature after refrigeration for—								
	1 day.	3 days.	6 days.	8 days.	10 days.	12 days.	16 days.	18 days.	24 days.
½ day	81	2	0	.....	0	0	0	.....	.....
1 day	92	35	0	.....	0	.....	.....	0	.....
2 days	62	5	0	0	0	0	0	0	0
3 days	81	2	0	0	0	0	0	0	0
4 days	55	3	0	0	0	0	0	0	0
5 days	70	32	14	7	0	0	0	0	0
6 days	94	21	10	0	0	0	0	0	0
7 days	69	60	51	10	1	0	0	0	0
9 days	[a] 31	[a] 27	[a] 23	25	0	0	.....	.....	.....

[a] Of these three lots of 100 pupæ each, 25, 17, and 16 pupæ, respectively, yielded adults July 8, or just before being placed in cold storage.

A total of 1,700 pupæ of various ages removed to normal temperature after refrigeration for 21, 27, 29, and 31 days were found to be dead.

TEMPERATURES OF FROM 38° TO 40° F.—A total of 52,604 pupæ were used in experiments to determine the effect upon pupæ of refrigeration at 38° to 40° F. An average of 1,860 pupæ of all ages were removed after refrigeration for 3, 4, 6, 7, 8, 10, 12, and 14 days. The number of pupæ for each age varied from 109 to 414 and averaged 234. The number of pupæ surviving refrigeration for from 3 to 14 days is recorded in Table IV.

TABLE IV.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration for from 3 to 14 days at 38° to 40° F.*

Age of pupæ on entering storage.	Number of pupæ yielding adults on removal to normal temperature after refrigeration for—							
	3 days.	4 days.	6 days.	7 days.	8 days.	10 days.	12 days.	14 days.
1 day	110	13	5	1	5	1	0	1
2 days	150	170	75	119	121	145	124	42
3 days	62	58	81	23	4	6	4	4
4 days	35	41	16	13	19	4	1	1
5 days	91	86	52	63	24	9	12	6
6 days	132	82	59	52	23	6	7	0
7 days	235	117	114	121	96	60	32	22
8 days	207	234	136	161	180	61	63	19

After refrigeration for 17 days only 3 out of 306 eight-day-old pupæ, 3 out of 384 seven-day-old pupæ, 3 out of 206 six-day-old pupæ, 1 out of 162 four-day-old pupæ, and 11 out of 374 two-day-old pupæ yielded adults, or only 21 out of 2,352 pupæ of all ages survived.

After refrigeration for 18 days only 9 out of 701 eight-day-old pupæ, 5 out of 250 seven-day-old pupæ, 1 out of 295 five-day-old pupæ, 1 out of 430 three-day-old pupæ, and 13 out of 400 two-day-old pupæ yielded adults; or only 29 out of 2,632 pupæ of all ages survived.

Nineteen days of refrigeration proved fatal to 1,911 pupæ of all ages except 2 out of 375 one-day-old pupæ. No living pupæ were found among 2,031 pupæ of all ages after refrigeration for 21 days, nor among 28,700 pupæ of all ages after refrigeration for 35 days.

TEMPERATURES OF FROM 40° TO 45° F.—In this experiment to determine the effect upon pupæ of temperatures ranging between 40° and 45° F., 8,800 pupæ from 1 to 10 days old were used. Each unit of pupæ contained 100 forms; hence, the numbers of pupæ yielding adults after refrigeration from 1 to 27 days as recorded in Table V represent the percentages of survival.

TABLE V.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration at 40° to 45° F. for from 1 to 27 days*

Age of pupæ on entering storage.	Number of pupæ yielding adults after removal to normal temperature after—									
	1 day.	3 days.	6 days.	8 days.	10 days.	12 days.	16 days.	18 days.	24 days.	27 days.
1 day	22	78	15	3	1	13	.....	.....	.....	.....
2 days	81	77	59	.....	24	.....	.....	3	.....	.....
3 days	69	91	1	1	2	0	.....	0	0	0
5 days	47	63	38	13	20	13	0	0	0	0
6 days	66	82	72	58	49	31	5	0	0	0
7 days	91	87	75	.....	60	57	.....	.....	.....	.....
8 days	55	60	44	54	27	16	4	1	1	1
9 days	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10 days	[a] 17	[a] 23	[a] 21	8	1	[a] 1	.....	0	.....	.....

[a] Besides these, 18, 28, 30, and 10 pupæ, respectively, yielded adults just before pupæ were placed in cold storage.

It will be noted that only 9 out of 300 pupæ survived refrigeration for 16 days, while only 4 out of 500 and 1 out of 500 refrigerated for 18 and 24 days, respectively, survived. Three hundred pupæ refrigerated for 31 days and 200 refrigerated for 34 days were found dead on removal.

TEMPERATURES OF FROM 49° TO 51° F.—Temperatures ranging between 49° and 51° F. and averaging about 50° have proved most interesting of all, as these appear to be very close to the point below which the insect's activities cease. This temperature was secured by use of an ordinary refrigerator 42 by 34 by 18 inches. During the period from May to July, 1914, 31,700 pupæ were used in an experiment to determine the effect of this temperature upon pupal development. Pupæ in 15 lots, of ages ranging from 1 to 8 days, and averaging 3,523 pupæ for each of the 8 days represented, were held in storage for two months before removal. Frequent observations were made but no pupæ completed their development and yielded adults in storage. On removal to normal temperature all of the 31,700 pupæ were found dead.

The second lot of 7,800 pupæ placed in storage when 5 days old yielded a few adults. Thus, 9 out of 7,800 yielded 1, 2, 2, 3, and 1 adult in storage after refrigeration for 20, 23, 44, 46, and 47 days. In other words, it took these 9 pupæ from 20 to 47 days to accomplish the development in refrigeration which at an outdoor temperature at that season, July, 1914, would have taken only from 4 to 5 days.

TEMPERATURES OF FROM 52° TO 56° F.—Ten larvæ pupating in a refrigerator held at 52° to 56° F. yielded 2 and 1 adult in storage after refrigeration for 38 and 52 days, respectively. The remaining 7 pupæ died.

TEMPERATURES OF FROM 54° TO 57° F.—Temperatures of from 54° to 57° F. were obtained by using an ordinary refrigerator 46 by 27 by 18 inches. A total of 22,700 pupæ were used varying in age from ½ to 9 days. Not less than 1,400 pupæ, or more than 3,500 pupæ of any age, were used. In Table VI are recorded the reactions of 3,100 one-day-old pupæ to these temperatures.

From the data in Table VI it will be noted that 54° to 57° F. is not in all cases fatal to pupal development, although a high mortality occurs. Each outward date represents 100 pupæ. As the heavy line extending diagonally across the table indicates the dates on which pupæ were removed from refrigeration, and as the normal pupal development is completed at this season of the year at Honolulu in from 9 to 12 days, the data prove that development continues at this temperature as evidenced, first, by the rate of emergence of adults after the pupæ are removed from refrigeration up to the thirtieth day of refrigeration, and, secondly, by the emergence actually occurring within storage on the thirty-first day and up to the thirty-seventh day of refrigeration. Thus development was wholly completed and emergence had taken place at this temperature among pupæ removed from refrigeration after 37, 38, and 39 days.

TABLE VI.—*Effect upon 1-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913*

Outward date.	Date and emergence of adults.																														
	August.				September.																										
	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Aug. 24	1	0	0	10	32	3																									
26		1	0	2	4	25	21																								
27							17	19	1																						
28								29	36																						
Sept. 3									1	0	0	15	14	1																
4												1	24	17																
5													1	19	12															
6														14	15															
7															6	16	4													
8													1	0	0	23	22	1												
9																	13	18												
10																	2	22	18											
12																		1	5	17	8									
13																	1	0	0	11	23	4								
14																				1	13	28								
15																			0	0	4	24	10							
16																						8	30	7						
17																						1	3	11	13					
18																						1	2	21	19					
19																								5	24	6				
20																									3	9	9			
21																									4	10	7	6		
22																									1	6	15	9	5	
23																									7	3	5	3	6	
24																									1	7	3	5	6	3
25																							1	0	3	2	5	3	9	11
26																										3	9	8	7	4	2
27																							1	0	0	1	6	2	2	1	3	3	2
28																		1	0	0	1	6	6	3	8	1	7	0	1	1		
29																									3	6	6	5	4	1		
30																									3	16	9	3	2	2	4	2	

Of 1,700 pupæ one-half day old when placed in refrigeration none emerged within storage until the twenty-fourth day of refrigeration, after which no data were secured. One hundred pupæ removed after 24 days of refrigeration produced 2, 27, and 5 adults 4, 5, and 6 days after removal, proving that even among these very young pupæ development was not completely arrested. Out of two lots of 100 pupæ each, 46 and 44 pupæ, respectively, refrigerated for 22 and 23 days, yielded adults 3, 4, and 5 days after removal from storage.

Data on file bring out an interesting fact that might be expected from the data in Tables VI and VII. The older the pupæ when placed in refrigeration, the quicker they develop and produce adults while in refrigeration. Thus in Table VII are recorded data on the development of six-day-old pupæ.

It will be noted that while a few one-day-old pupæ require a minimum of 31 days of refrigeration for development, 2 six-day-old pupæ com-

pleted their development and produced adults in storage on the fifth day of refrigeration, and that thereafter emergence of adults continued until all living pupæ yielded adults in storage by the end of the sixteenth day of refrigeration except 2, which yielded adults on the fourth day after removal after 16 days of storage. Data on 1,900 eight-day-old pupæ show that from 1 to 5 pupæ among each of 14 different lots of 100 completed their development and yielded adults on the second day of refrigeration, that an average of 43.5 per cent of 14 lots yielded adults on the third day, and that emergence of adults was completed by the seventh day except in one instance where 2 pupæ yielded adults in storage on the ninth and tenth days of refrigeration.

TABLE VII.—*The effect upon six-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913*

Outward date.	Date and emergence of adults.															
	August—							September—								
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10
Aug. 24			57	6													
26			1	45	29				1								
27				2	37	0	22	0	1								
28					4	0	67										
Sept. 3					1	0	10	8	19	13	0	3					
4							13	13	13	7	11						
5			0	0	0	0	13	13	12	12	12	2	3				
6				1	1	0	7	12	16	15	17	10	4				
7			1	0	1	0	11	10	13	9	19	7	2	0	0	0	2
8			1	1	0	0	13	10	13	13	11						
9			0	2	2	0	11	5	9	15	10	2					
10				1	0	0	13	21	13	9	18	3	2				
12			0	0	0	0	11	6	13	10	13	1	2	3			

Data on file covering observations on 1,600, 1,400, 1,700, 2,200, 3,300, 4,000, 3,100, 3,500, and 1,900 pupæ, 1, 2, 3, 4, 5, 6, 7, 8, and 9 days old, respectively, show a steady increase in the pupæ completing their development and yielding adults in refrigeration, and their tabulation shows a transition from the condition in Table VI through that of Table VII to the condition set forth for eight-day-old pupæ. One lot of two-day-old pupæ left in storage for 37 days yielded adults almost daily between the twenty-sixth and thirty-fifth days of refrigeration. Eight lots of three-day-old pupæ left in storage from 32 to 39 days yielded adults between the twenty-third and thirty-third days of refrigeration and none thereafter. Fourteen lots of four-day-old pupæ in storage from 25 to 39 days yielded adults between the sixteenth and twenty-seventh days of refrigeration and none thereafter. Eight lots of seven-day-old pupæ in storage from 13 to 21 days yielded adults between the second and eleventh days of refrigeration and none thereafter.

CONCLUSION

From the data secured during experimental work reported on the foregoing pages, including observations on 173,318 pupæ of the Mediterranean fruit fly (*Ceratitis capitata* Wied.), it appears that no pupæ survive refrigeration for longer periods than is necessary to cause the death of eggs and larvæ in host fruits held at corresponding temperatures.

About 50° F. is the critical point below which development can not take place and below which death will follow if refrigeration is continued sufficiently long. At 49° to 51° only 9 out of 39,500 pupæ yielded adults in refrigeration 20 to 47 days after the inward date, while 3 out of 6 held at 52° to 56° yielded adults in refrigeration 38 to 52 days after the inward date. Many pupæ can complete their entire development in refrigeration at 54° to 57°, while higher temperatures, not considered here, merely retard development without causing noticeable mortality.

Pupæ can not withstand temperatures below 50° F. for prolonged periods of time. Only 3 and 1 pupa survived refrigeration for 8 and 9 days, respectively, at 32°, while none of 4,500 pupæ survived 10 days at this temperature. Refrigeration at a temperature averaging 34°, but ranging between 33° and 36°, proved fatal after the seventeenth day; 6,017 pupæ refrigerated at this temperature for 18 and 25 days yielded no adults, while the number to yield adults after refrigeration for 14 and 17 days was very small. No pupæ survived refrigeration at 28° to 40° but averaging 36°, for more than 10 days. A temperature of 38° to 40° proved fatal after the nineteenth day; 30,731 pupæ refrigerated for from 21 to 35 days failed to yield adults on removal to normal temperatures. After refrigeration at 40° to 45° pupæ from each of two lots removed after refrigeration for 24 and 27 days, respectively, yielded adults; 500 pupæ removed after refrigeration for from 31 to 34 days proved to be dead.

It does not seem safe to conclude that the age of the pupa has a direct bearing upon its ability to withstand the more ordinary ranges of cold-storage temperatures.

EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM

By J. J. WILLAMAN and R. M. WEST, *Assistant Chemists, Agricultural Experiment Station of the University of Minnesota*

[In collaboration with F. S. Harris, *Agronomist, Utah Agricultural Experiment Station;* L. E. Call, *Agronomist, Kansas Agricultural Experiment Station;* and Beyer Aune, *Superintendent, Belle Fourche Experiment Farm, Newell, S. Dak.*]

INTRODUCTION

The present experiments are a continuation of those carried out in 1914 (10)[1] on sorghum (*Sorghum vulgare*). In the latter a correlation was sought between the soil conditions, especially the supply of nitrogen, and the amount of the cyanogenetic glucosid (dhurrin) in the sorghum. It was found that on fertile soils nitrogenous fertilizer has no appreciable effect, but on poor soil added nitrogen may increase the amount of hydrocyanic acid, though only to a small extent. Since the evidence indicated that climate and variety may be more important factors than soil nitrogen in determining the amount of the glucosid in this plant, experiments were carried out during 1915 to study the effect of climatic conditions. It was thought that conditions of high or low temperature, much or little available water, slow or rapid growth, might affect the metabolism of sorghum sufficiently, not only to show the causes of the varying amount of dhurrin, but also to throw some light on the physiological function of this glucosid.

EXPERIMENTAL WORK

Seeds of two varieties of sorghum were obtained. One was Early Amber, grown in Minnesota, and is designated in these experiments Variety N. The other was Southern Cane, a variety similar to the first, but grown in Missouri. It is designated Variety S. In order to secure as widely varying climatic conditions as possible, one-twentieth-acre plots of each variety were grown at four different State experiment stations. A brief description of each plot follows:

1. University Farm, St. Paul, Minn. Very fertile, black loam, fair drainage. Planted on June 3; sprouted on June 12; cultivated twice. Season very cold and wet; sorghum three or four weeks behind the normal in development; did not reach maturity, but was killed by frost in the soft dough stage.

2. Agricultural Experiment Station, Logan, Utah. Irrigation farming. Plots on McNiel farm, North Logan; the two varieties alternated with beans; soil a clay loam, rich in manure. Planted May 15; appeared aboveground on June 1; irrigated on July 9 and August 11; cultivated on June 10, June 17, July 1, July 13, and August 17. Rainfall up to June 10 was abnormally high, which kept the soil cold and retarded growth of crops. During the rest of the season optimum moisture content of soils

[1] Reference is made by number to "Literature cited," p. 272.

Journal of Agricultural Research, Dept. of Agriculture, Washington, D. C.
dr

Vol. VI, No. 7
May 15, 1916
Minn.—9

obtained. Sorghum made slow growth; leaves yellowish for first seven or eight weeks.

3. Agricultural Experiment Station, Manhattan, Kans. Plots grown on "creek bottom land," broken from native sod in 1913; drainage poor. Planted on June 15; appeared aboveground on June 22; cultivated twice. Sorghum 30 days slower in maturing than usual, owing to excessive rains.

4. Belle Fourche Experiment Farm, Newell, S. Dak. Dry farming. Planted on June 10; appeared aboveground on June 26; cultivated on July 22 and August 7; harvested on October 12. Season cold and wet; rainfall far above normal.

5. Belle Fourche Experiment Farm, Newell, S. Dak. Irrigation farming. Planted on May 31; appeared aboveground on June 26; cultivated on July 23 and August 10; irrigated on August 17; harvested on September 16, when plants were just headed out.

From the time when the plants were from 20 to 30 cm. in height, samples were taken every 10 days. They were usually cut between 9 and 12 a. m., although it has been found that the time of day makes no difference in the amount of hydrocyanic acid present. Plants were selected which represented the average of the plot on that date. For the first sample the whole plant was cut into 1-inch lengths and packed into a 600 c. c. friction-top tin can with 20 c. c. of 3 per cent alcoholic sodium hydrate and 2 c. c. chloroform for preservatives and sent to the Minnesota laboratory for analysis. For the other samples the leaves were cut off where they join the sheath, and the leaves and stalks were packed and analyzed separately. The weight of leaves and of stalks in the total sample cut was recorded. From the fourth sample on, cans of 1,600 c. c. capacity were used. An alkaline preservative was used so as to prevent any possible loss of hydrocyanic acid set free by enzymic activity. Alcohol instead of water was used as a solvent for the alkali, because it penetrates the plant tissues more readily. The chloroform prevented any fermentative changes. In the case of the South Dakota, Kansas, and Utah samples, from two to five days elapsed from the time the samples were cut till they were analyzed. In the case of the Minnesota samples, the fresh material was analyzed. In order to test the efficiency of the preservative, several samples from the Minnesota plots, representing the various stages of maturity of the samples outside of Minnesota, were analyzed for hydrocyanic acid before and after storage in cans, with the results given in Table I.

TABLE I.—*Efficiency of an alkaline preservative in preventing loss of hydrocyanic acid in sorghum*

Preservative treatment.	Percentage of hydrocyanic acid in dry matter.	
	Fresh.	Preserved.
Preserved for four days with alcoholic sodium hydroxid and chloroform	0.019	0.020
Do	.026	.029
Preserved for eight days with alcoholic sodium hydroxid and chloroform	.009	.009
Do	.016	.019

The differences noted are within the limits of accuracy of sampling and analyzing; hence, this method of preservation can safely be used on sorghum plants at least through the stages of maturity represented in these experiments.

About 50 gm. of the sample, after thorough mixing and fining with a knife, were used to determine the percentage of dry matter. For the determination of the hydrocyanic-acid content, from 50 to 70 gm. were ground in a food chopper, placed in an 800 c. c. Kjeldahl flask, together with 250 c. c. of 5 per cent tartaric acid, and distilled slowly into 10 c. c. of 2 per cent sodium hydroxid until the distillate was nearly 100 c. c. This completely hydrolizes the dhurrin and carries the hydrocyanic acid over into the alkaline distillate. The latter was made to 100 c. c. and aliquots used for the determination of hydrocyanic acid according to the method of Viehoefer and Johns (9). This method was found to be easier and more accurate than the thiocyanate method used in 1914.

The complete analytical results appear in Table II. The figures for the amount of hydrocyanic acid in the whole plant were computed from the relative proportion of leaves and stalks in each sample.

TABLE II.—*Hydrocyanic-acid content of sorghum from the various experimental plots*

[The percentage of hydrocyanic acid is reported on a dry-matter basis]

Plot and sample No.	Date of sampling.	Age of plants since sprouting.	Plot N.				Plot S.			
			Height of plants.	Percentage of hydrocyanic acid.			Height of plants.	Percentage of hydrocyanic acid.		
				Stalks.	Leaves.	Whole plant.		Stalks.	Leaves.	Whole plant.
Minnesota:		*Days.*	*Cm.*				*Cm.*			
1	July 15	33	25	.....	.....	0.114	25	.....	.....	0.079
2	July 24	42	44	0.026	0.031	.028	48	0.028	0.035	.032
3	Aug. 3	52	68	.018	.021	.019	65	.015	.031	.026
4	Aug. 13	62	90	.012	.008	.009	88	.023	.011	.016
5	Aug. 24	73	135	.000	.004	.002	133	Trace.	.007	.003
6	Sept. 3	83	160	Trace.	.004	.001	160	.000	.004	.001
7	Sept. 13	93	188	.....	.001	Trace.	192	.....	.002	Trace.
8	Sept. 23	103	205	.....	.....	.....	206	.....	.003	Do.
Utah:										
1	July 19	49	36	.....	.....	.034	36	.....	.....	.040
2	July 29	59	56	.028	.013	.019	59	.039	.025	.031
3	Aug. 7	68	78	.019	.023	.021	84	.026	.032	.029
4	Aug. 18	79	120	Trace.	.022	.009	116	.007	.034	.020
5	Aug. 28	89	161	.000	.026	.008	160	Trace.	.041	.016
6	Sept. 7	99	174	.....	.....	.....	175	.....	.....	.....
7	Sept. 17	109	190	.....	.....	.....	192	.....	.....	.....
Kansas:										
1	July 16	24	18	.....	.....	.016	26	.....	.....	.014
2	July 27	35	75	.000	.017	.008	95	.000	.030	.014
3	Aug. 5	44	137	.000	.007	.003	150	.000	.020	.008
4	Aug. 16	55	200	.....	.....	.....	210	.....	.....	.....
5	Aug. 25	64	260	.....	.....	.....	255	.....	.....	.....
Dakota (dry farming):										
1	July 26	30	33	.....	.....	.020	33	.....	.....	.030
2	Aug. 5	40	61	.004	.013	.011	61	.009	.021	.016
3	Aug. 14	49	91	.000	.000	.000	86	.000	.008	.004
4	Aug. 24	59	142	.000	.004	.002	137	.000	.006	.002
5	Sept. 4	70	189	.....	.....	.....	187	.....	.....	.....
6	Sept. 14	80	282	.....	.....	.....	287	.....	.....	.....
Dakota (irrigation):										
1	July 26	30	35	.....	.....	.009	37	.....	.....	.026
2	Aug. 5	40	51	.004	.008	.006	51	Trace.	.009	.005
3	Aug. 14	49	96	.000	Trace.	Trace.	91	.000	Trace.	Trace.
4	Aug. 24	59	134	.000	...do...	...do...	134	.000	.001	Do.
5	Sept. 4	70	193	.....	.....	.....	190	.....	.....	.....

In figure 1 the percentages of hydrocyanic acid in the whole plant are plotted against the age in days. No noteworthy differences were noticed when the height of the plants was used instead of the age in days.

Figure 2 represents the growth curve of the various plots, where the height in centimeters is plotted against the age in days since sprouting.

In order to study the relation between climatological factors and the content of hydrocyanic acid, figures 3 and 4 were constructed. In

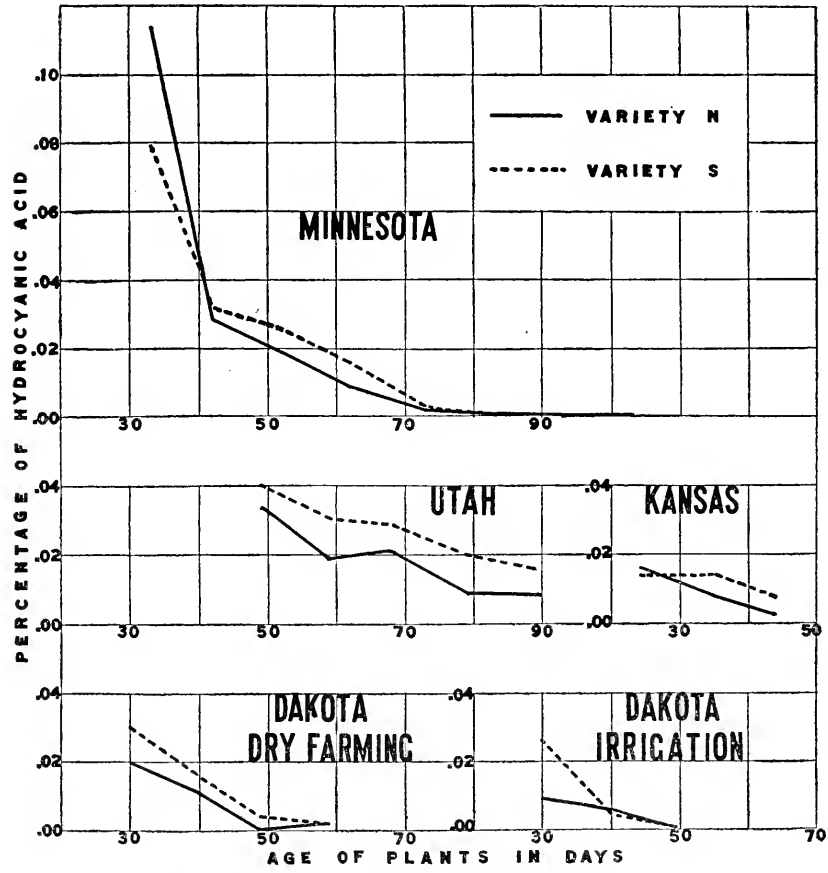


FIG. 1.—Curves showing the hydrocyanic-acid content of sorghum on the various plots. (Percentage of hydrocyanic-acid computed to dry-matter basis.)

figure 3 are plotted first the precipitation (in inches) during 15-day intervals; second, the temperature (degrees Fahrenheit), using averages for 10-day periods, and, third, the mean relative humidity (percentage) at 6 a. m., all for the five months May to September, inclusive. In figure 4 the history of each plot for the season is shown and includes the rainfall, temperature, and hydrocyanic-acid curves on the same graph. The dates for planting, sprouting, appearance of seed panicles, and irrigations are also shown.

DISCUSSION OF RESULTS

The season of 1915 furnished some excellent extremes in weather conditions for this experiment. Figure 2 shows that, as regards temperature, the two more southern States, Kansas and Utah, form one pair, and South Dakota and Minnesota another, with approximately 10 degrees difference between them during the growing season. Of the two warmer stations, Utah had a low rainfall, and irrigation was resorted

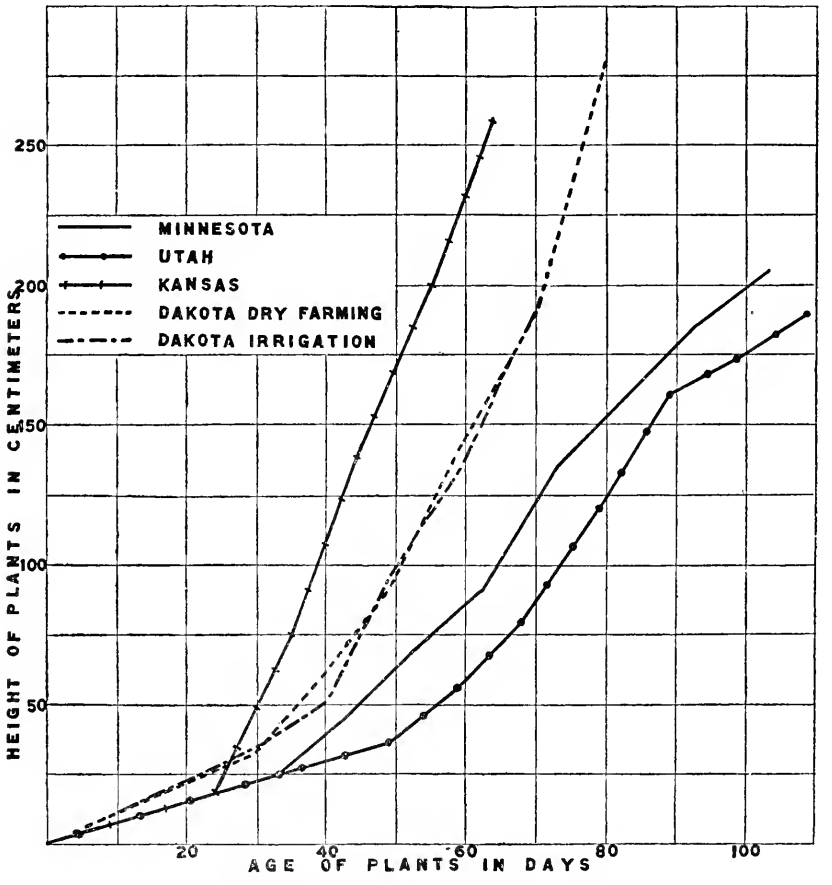


FIG. 2.—Curves showing the rate of growth of the sorghum on the various plots.

to; while Kansas had a very abundant rainfall, resulting even in flood conditions during May, June, and July. The two more northern stations had about the same rainfall for the first three months of the experiment, but during the period when the samples were taken the rainfall of South Dakota dropped below that of Minnesota. This was particularly the case during August. The 1915 rainfall of South Dakota was above normal, and as a result the plot on irrigation ground was irrigated only once.

The five plots differed rather widely in their rate of growth, as is shown in figure 4. The Utah plants were 48 days old before attaining the height required for first sample. During this time they looked yellow and unthrifty, owing to excess moisture and cool soil. Subsequently

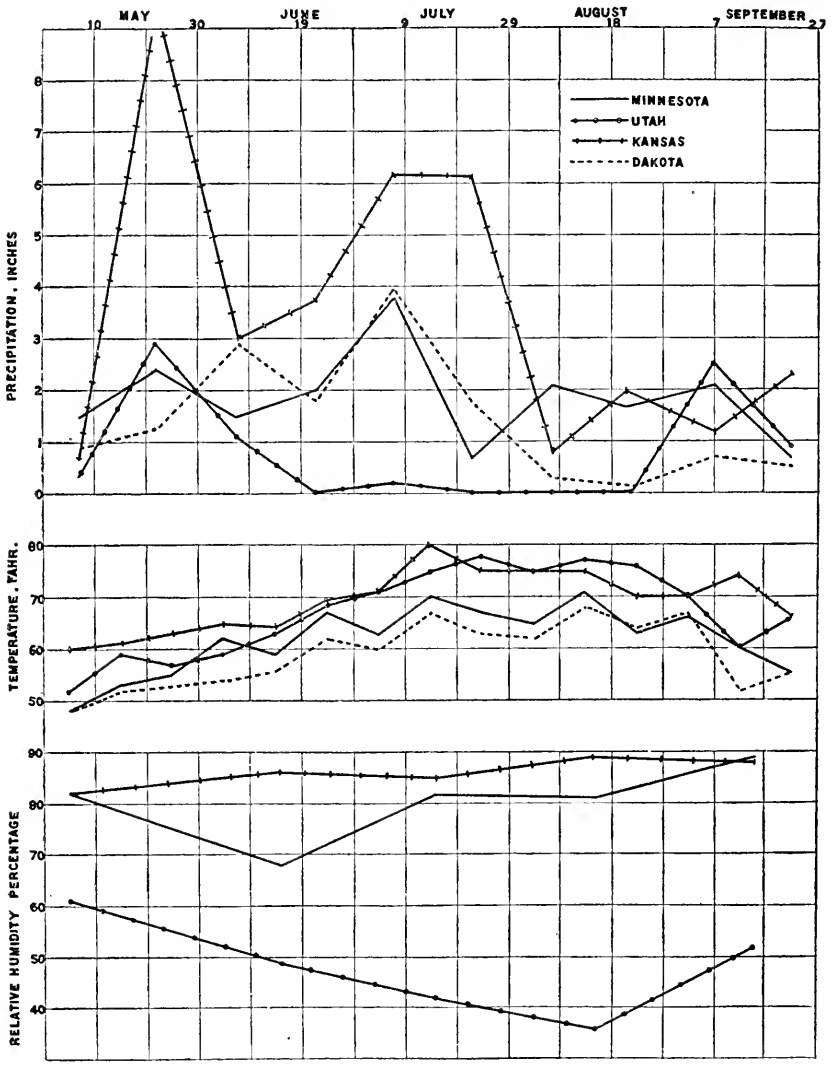


FIG. 3.—Curves showing the precipitation, temperature, and humidity relations at the various experiment stations during the growing season of 1915.

the plots grew nearly as fast as those at the other stations and gave a higher yield of dry cane at the end of the season than did the South Dakota plots, although the latter grew much taller. The Kansas plots grew the most rapidly.

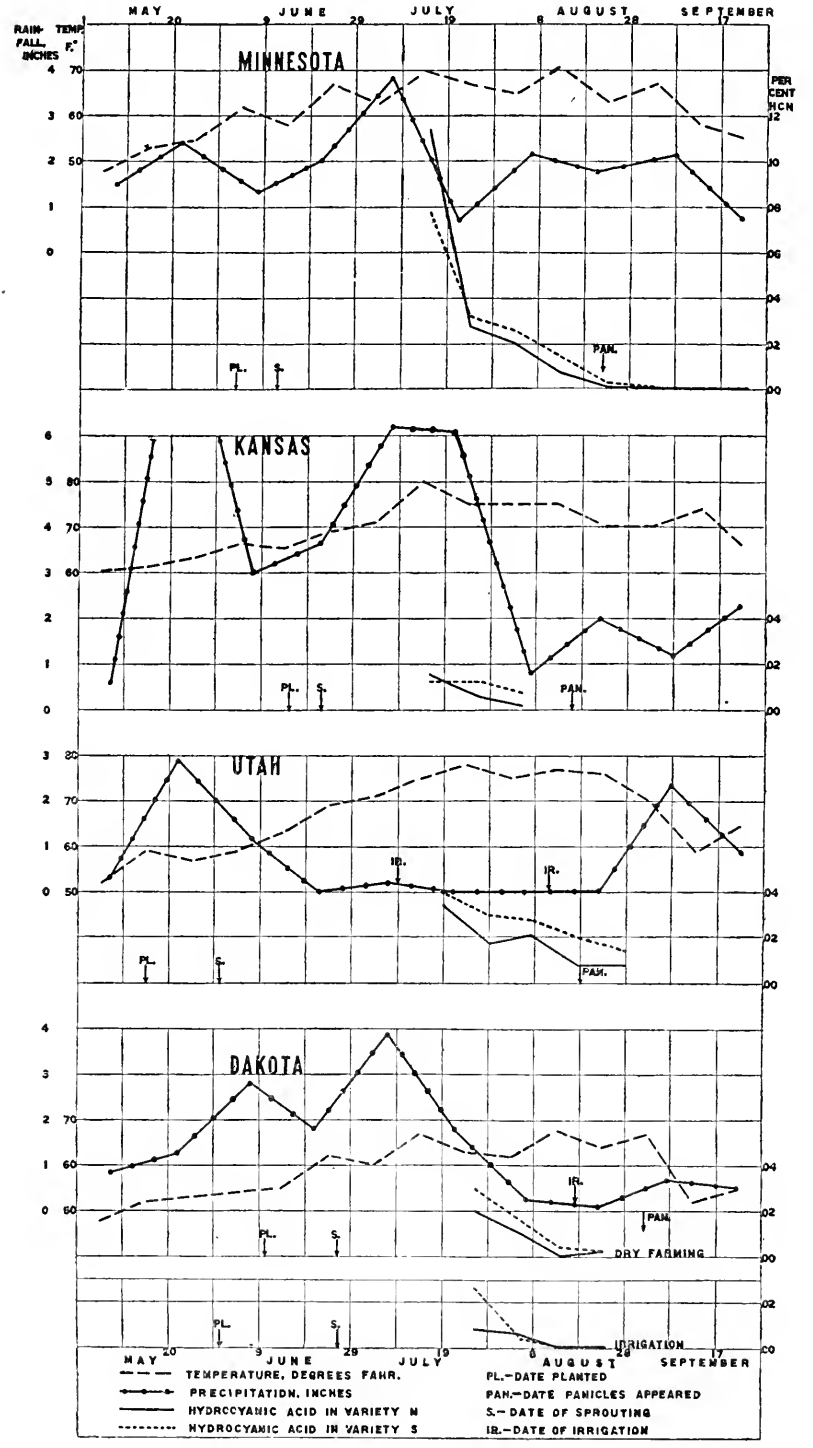


FIG. 4.—Curves showing the contemporary climatic conditions at the various plots, together with crop data and hydrocyanic-acid content.

Accompanying these various conditions were also widely differing amounts of hydrocyanic-acid glucosid. How the correlations between these two may be explained will depend upon the function assigned to the dhurrin in sorghum. Various uses have been attributed to glucosids in plants, as (*a*) a protection against bacteria and other enemies by means of the poison set free when some glucosids are hydrolyzed; (*b*) a reserve food material in the plant; (*c*) the inactive form of a stimulating hormone (2) set free when necessary by a glucosidase; (*d*) a harmless compound absorbing injurious products of metabolism; (*e*) an inactive storage of "respiratory pigments," and other uses.[1] Hydrocyanic acid itself is thought by some investigators to be a necessary intermediate product in protein formation (6, 8). As such, it is probably rather transitory in the plant, and seldom occurs free in any appreciable amount.

A discussion of each factor which might have any bearing on the cause of the variations in cyanid content, or throw any light on the function of dhurrin in sorghum, follows.

1. HUMIDITY.—It is hard to perceive how the relative humidity might have any direct bearing on the quantity of dhurrin produced. The humidity affects primarily the rate of transpiration, and this in turn might influence the rate of growth. The latter factor is considered in the next paragraph. The interesting thing to note in the humidity curves in figure 2 is the fact that the Utah curve shows a decrease during a period of decreasing precipitation, which is natural, but the Kansas and Minnesota curves show an increase during periods of decreasing precipitation. It is possible that this very low humidity in Utah caused a rate of transpiration too high for the best development of the plants, and their growth was retarded accordingly. When the humidity was lowest, in July and August, the plots received their two irrigations. Following these the growth was more rapid. If the humidity affects the amount of glucosid at all, it is by means of its effect on the nutrition and growth of the plant.

2. MOISTURE SUPPLY.—As mentioned above, there are among the four stations one having very high, one with very low, and two with medium rainfall. Two plots at the last-named stations were under irrigation and one under dry-farming methods of cultivation. In the data as a whole there is no evident correlation between the amount of the glucosid and the moisture supply for the five months. Arranging the stations in the order of their moisture supply, they are Kansas, Minnesota, South Dakota irrigation, South Dakota dry farming, and Utah; while arranged in the order of their cyanid content they are Minnesota, Utah, Kansas, and South Dakota dry farming the same, and South Dakota irrigation. However, by a closer examination of the curves for

[1] For a complete discussion of the function of glucosids in plants see Armstrong, E. F., The Simple Carbohydrates and the Glucosids. Ed. 2, p. 125–133. London, New York, 1912.

each plot, the following examples tending to show that high water supply is often accompanied by a low cyanid content are discernible: (1) The normal hydrocyanic-acid curve for sorghum during the first two-thirds of its growth is a smooth curve, with a steady decrease in the acid. The Utah curve is an exception to this. In this plot there was for several weeks very rapid transpiration of water, owing to low humidity; hence, the plants and soil were reduced nearly to the minimum water requirement. Shortly after the first irrigation the hydrocyanic acid is seen to be on a normal decline. Twenty days after this irrigation, however, the plots had become comparatively dry again, and the hydrocyanic acid shows a less decrease in variety S and an actual increase in variety N. The second irrigation was followed by another decline in hydrocyanic acid. By the latter part of August the need of water was once more felt, and the cyanid in variety N, at least, had ceased to decrease. (2) The curve for the South Dakota dry-farming plot also shows an abnormality in that the last part of it has an upward turn in the case of variety N. It is possible that this may be due to the smaller supply of moisture available at this time. (3) In the two South Dakota plots, both received the same amount of rain; one was irrigated once and the other, being cultivated by dry-farming methods, had a larger reserve supply of water. This would apparently give them about the same amount of water supply, except for the fact that the irrigation was a heavy one, and the heavy rains during May, June, and July disturbed the usual dry-farming condition of the soil. Assuming that the irrigated plots did have more water available, it will be seen that they also contained a less amount of hydrocyanic acid. (4) On analyzing some sorghum plants grown in pots in the greenhouse, they were found to contain no hydrocyanic acid. A few weeks later some larger plants from this same group, growing in drier soil, owing to lack of care in watering and to a larger demand made by the plants, were found to contain some of the acid. There appears, therefore, to be a relation between the supply of water and the amount of dhurrin present. This may be explained on the hormone theory With a liberal supply of water, other things being equal, the plant's means for growth are adequate and it needs less glucosid. With a decreasing water supply, however, the plant may need the hormone stimulus for growth, and more glucosid is produced. Although, as shown by Briggs and Shantz (5), sorghum has a lower water requirement than most cultivated plants, it is no doubt affected by changes in the supply of moisture.

3. TEMPERATURE.—No correlation has been found between the content of dhurrin in sorghum and variations in temperature, at least for the range of temperatures which obtained during this experiment. The increase in hydrocyanic acid which sometimes occurs when plants are frosted may be due to disturbed enzym balance.

4. Rate of growth.—The Kansas and Utah plots present extremes in rate of growth and thriftiness of the sorghum plants; and they also present cases of relatively low and high hydrocyanic-acid content, respectively. The cane on the Minnesota plots grew more slowly than that from South Dakota, and it also contains a very much higher hydrocyanic-acid content. During the first four or five weeks on the Minnesota plots the plants grew very poorly, the weather being cold and damp. The plants were yellow and uneven in height, similar to those obtained from Utah. The samples from these two stations were by far the highest in hydrocyanic acid. In fact, the percentage in the first Minnesota sample, variety N (0.114 per cent), is the highest ever observed in the authors' experience with sorghum. In the Minnesota samples of 1914 those grown on the poorer sandy soil were the higher in cyanid. These examples, together with one furnished by Avery (3), show that some significant relation may exist between poor conditions of growth and high dhurrin content. In opposition to this, however, is the finding of Alway and Trumbull (1) that the yellower plants in a field contained a smaller amount of the acid. Balfour (4) found more in plants infested with *Aphis sorghi* than in others not so affected. If these facts are now applied to the various theories mentioned above, as to the function of glucosids, some of the possibilities are as follows: (1) If this particular glucosid is a food storage, it is difficult to see how it could exist in largest quantities in the unhealthy, poorly nourished, slow-growing plants. (2) If the constituents of the glucosid act as stimulating hormones when set free by an enzym, it is possible that when conditions of growth are poor more of the glucosid is produced. (3) If the glucosid is an absorber of harmful products of metabolism under disturbed metabolic conditions, an excess of hydrocyanic acid might be produced. Of these three the authors believe the second to be the most tenable for dhurrin, according to the available evidence on this question.

5. Variety.—The most striking phenomenon in this experiment is the fact that Variety S has consistently a greater amount of hydrocyanic acid than Variety N. That varietal difference is very important was brought out also in the 1914 experiments. In fact, the authors are confident that the most marked and constant differences in the hydrocyanic-acid content of various sorghum plants will be found to be due to variety rather than to external conditions. A comparative study of the glucosid content of all varieties of sorghum would be interesting and valuable.

6. Distribution in the plant.—The foregoing discussion has been based on the dhurrin content of the whole plant. As is seen from Table II, the distribution of the glucosid between stalk and leaves in the different plots is variable. There is in every instance a more rapid decrease in the stalks than in the leaves, but the comparative rate of decrease varies. The Minnesota and Utah plots had the highest amount in the stalks and also had the slowest growth and the thinnest stalks. The Kansas and

the South Dakota plots, on the other hand, had little or no cyanid in the stalks and had the most rapid growth. The Kansas stalks were very heavy and succulent; they had developed very rapidly; and they contained no cyanid whatever. The significance of this is not clear.

7. DAILY VARIATION.—In order to compare the glucosid content in sorghum with Treub's findings (7, 8) that in *Pangium edule* there is a daily variation in glucosid content with a maximum about midday, some analyses were made at sunset and sunrise of succeeding days, with the results given in Table III.

TABLE III.—*Variation in the glucosid content of sorghum at different parts of the day*

Variety.	Part of plant.	Percentage of hydrocyanic acid.	
		Evening.	Morning.
Variety N	Leaves	0.015	0.012
Do	Stalks	.013	.014
Dakota Amber	Leaves	.020	.023
Do	Stalks	.023	.031

There seems to be no constant variation in sorghum between night and day. This lends support to the view that dhurrin is not a food storage.

Although other factors have important bearing on the growth and health of plants, those discussed above are the most readily measured and, hence, best used as bases for comparison between widely separated stations. It is realized that determinations of soil moisture at various times throughout the growing season would give a much more accurate idea of the available moisture than precipitation measurements. As regards soil, each plot was grown on soil which has produced good crops in the past and was cultivated according to the customary methods for sorghum at those stations. Since the 1914 experiments showed that soil is a minor factor in affecting the hydrocyanic-acid content of sorghum, the ignoring of this factor in the above comparisons is justified.

SUMMARY

Two varieties of sorghum, Southern Cane and Early Amber, were grown on plots in Minnesota, Utah, Kansas, and South Dakota under widely different climatic and cultural conditions. The amount of the glucosid dhurrin in each plot varied considerably. The following correlations relative to the amount of glucosid were found to exist.

(1) Unhealthy plants usually contain more hydrocyanic acid than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, to insect attack, or to other causes. It is possible that under such conditions the plant produces more glucosid for the sake of the stimulating hormones in it.

(2) The apparent effect of humidity and temperature on the amount of cyanid in sorghum is probably due to the indirect effect on the rate of growth.

(3) Adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic-acid content. This is probably due to the need of glucosid stimulation when the water supply becomes low.

(4) The character of the growth of the plant affects the distribution of dhurrin between leaves and stalks, there being a proportionately smaller amount in the thick, heavy stalks than in the slender ones.

(5) There is no consistent daily variation in the amount of dhurrin, which argues against the functioning of this glucosid as a food storage.

(6) Of the two varieties used in this experiment, the Southern Cane in every plot but one had a higher content of hydrocyanic acid than the Early Amber. Varietal difference is probably of more weight in determining the amount of hydrocyanic acid in sorghum than are the conditions of growth.

LITERATURE CITED

(1) ALWAY, F. J., and TRUMBULL, R. S.
1910. On the occurrence of prussic acid in sorghum and maize. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt., 1909, p. 35–36.

(2) ARMSTRONG, H. E., and ARMSTRONG, E. F.
1911. The function of hormones in regulating metabolism. *In* Ann. Bot., v. 25, no. 98, p. 507–519.

(3) AVERY, Samuel.
1902. Laboratory notes on poison in sorghum. *In* Jour. Comp. Med. and Vet. Arch., v. 23, no. 11, p. 704–706.

(4) BALFOUR, Andrew.
1904. Cyanogenesis in sorghum vulgare. *In* 1st Rpt. Wellcome Research Lab. Gordon Mem. Col. Khartoum, [1903/04], p. 46–48.

(5) BRIGGS, L. J., and SHANTZ, H. L.
1914. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1–63, pl. 1–7.

(6) RAVENNA, Ciro, and ZAMORANI, Mario.
1909. Nuove ricerche sulla funzione fisiologica dell' acido cianidrico nel Sorghum vulgare. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v. 18, sem. 2, fasc. 8, p. 283–287.

(7) TREUB, Melchior.
1907. Notice sur "l'effet protecteur" assigné á l'acide cyanhydrique des plantes. *In* Ann. Jard. Bot. Buitenzorg, v. 21 (s. 2, v. 6), pt. 1, p. 107–114, pl. 3–4.

(8) ———
1909. Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. III. *In* Ann. Jard. Bot. Buitenzorg, v. 23 (s. 2, v. 8), pt. 1, p. 85–118, pl. 18–23 (col.).

(9) VIEHOEVER, Arno, and JOHNS, C. O.
1915. On the determination of small quantities of hydrocyanic acid. *In* Jour. Amer. Chem. Soc., v. 37, no. 3, p. 601–607.

(10) WILLAMAN, J. J., and WEST, R. M.
1915. Notes on the hydrocyanic-acid content of sorghum. *In* Jour. Agr. Research, v. 4, no. 2, p. 179–185, 2 fig. Literature cited, p. 185.

EGG AND MANNER OF OVIPOSITION OF LYCTUS PLANICOLLIS[1]

By THOMAS E. SNYDER,
Assistant in Forest Entomology, Bureau of Entomology

HISTORICAL SUMMARY

The so-called "powder-post" injury to seasoned wood products is widely distributed over the world. Of the various beetles causing this type of injury, species of the genus Lyctus Fab. are by far the most important. While these beetles and their damage have an extensive literature, the place and manner of oviposition have remained obscure. Heeger (3),[2] in 1853, described and figured the egg, larva, and pupa of a beetle attributed to a European species, *Lyctus pubescens* Panzer. Dugès (1),[3] in 1883, described and figured the larva, pupa, and adult of *L. planicollis* Le Conte (?), proving that Heeger was in error in ascribing the larva he figured to the genus Lyctus. Xambeu (7), in 1898, described the egg and manner of oviposition of *L. linearis* Goeze (*canaliculatus* Fab.). Recently the eggs of the native species, *L. planicollis* of the southern United States, have been found by the writer. This egg is very unlike that described and figured by Heeger as the egg of *L. pubescens*, and it differs from the egg of *L. canaliculatus* as described by Xambeu, being of a most unusual type for Coleoptera.

The following brief notes on the mating and oviposition of the southern species (*L. planicollis* Le Conte) were made on material being reared either at Washington, D. C., or at Falls Church, Va., in buildings kept dry and at a temperature above freezing.

LIFE CYCLE

MATING

The beetle passes the winter in the larval stage, but in cold weather the larvæ are more or less dormant and infested stock may consequently pass unnoticed. Mating takes place and the eggs are deposited soon after the adult beetles emerge from the wood in the spring. At Washington, D. C., and Falls Church, Va., the first adults emerged from infested wood in rearing cages during the last part of February and first part of March,

[1] The specimens on which this paper is based were identified by Mr. W. S. Fisher, Specialist on Forest Coleoptera, of the Branch of Forest Insect Investigations, Bureau of Entomology.

[2] Reference is made by number to "Literature cited," p. 276.

[3] According to Dugès, the material on which his paper was based had been determined by two different authorities as *Lyctus planicollis* Le Conte (of southern U. S.) and *carbonarius* Waltl. (of Mexico and Florida). Dugès refers to the species as *planicollis* in the title and *carbonarius* on the plate. Hopkins (5, p. 134) states that *L. carbonarius* is evidently distinct from *L. planicollis*, and therefore Dugès's specimens are *L. carbonarius*.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dq

Vol. VI, No. 7
May 15, 1916
K—32

in 1914 and 1915. At Baltimore, Md., adults of this species emerged from an infested oak table in a heated building as early as January 12, 1916. General emergence at Falls Church, Va., however, did not begin until about the middle of April, 1914 and 1915. The period of maximum activity is from the last of April to the first part of June. The last adults emerged during the first part of July. Mating occurred commonly during May, in 1915.

OVIPOSITION

Oviposition began a few days after mating and was observed to take place principally during the middle of May, in 1915. On May 24, 1915, many beetles were observed on radial sections of wood with their ovipositors deeply inserted into the open ends of pores or large longitudinal vessels in the wood, but the first eggs were not found till June 1, 1915.

The beetles seem to prefer to oviposit on those sections of seasoned sapwood where the open ends of pores are most numerous. These pores are especially prominent in "ring-porous" woods such as hickory, ash, and oak, which are also the species most subject to attack by Lyctus beetles. No eggs were observed on the surface of the wood, but all that were found were in these pores.

The females remain for several minutes with the ovipositor in the pore, and the process is repeated at several places. The female usually assumes a position in which the body is parallel to the pore and the ovipositor is either curved down and bent forward into the pore underneath the body or projected directly into the open end of the pore. However, the ovipositor, which is long and flexible and reaches from the end of the body to the thorax when extended forward, can be projected in any direction. At the extremity of the ovipositor are two laterally placed palpi. In the process of inserting the ovipositor into the pores, there is a considerable preliminary period of thorough examination with these palpi of all parts of the pore before an egg is laid. Two or more eggs are usually laid near together in each pore utilized. Each female deposits eggs in several pores.

THE EGG

The egg (Pl. XXVIII, fig. 1) is cylindrical, rounded at the ends, and has a slender strand or process attached to the cephalic pole. It is whitish in color, somewhat shiny, 1 mm. in length with the strand attachment, 0.75 mm. in length without this process, and 0.175 mm. in width. This process or strand is somewhat similar to that of the eggs of certain parasitic Hymenoptera—that is, parasites of the cotton boll weevil in the families Eurytomidae and Encyrtidae (6, p. 49–51, pl. 2), but this is the only instance known to the writer of such a process on the eggs of Coleoptera. The egg has a granular appearance (Pl. XXVIII, fig. 2), and at the end which terminates in the process there is an area marked with parallel, longitudinal striæ (Pl. XXVIII, fig. 4). The egg of *L. linearis*

(*canaliculatus*), as described by Xambeu, is very different from the egg of *L. planicollis*, since no mention is made by Xambeu of either the strand attachment or the area of longitudinal striæ, which are unusual characters in the egg of a beetle.

The end with the process (the cephalic pole) leaves the ovipositor last,[1] and this strand may possibly be attached by the ovipositor to the pore contents. The larva does not occupy much more than half the length of the egg (Pl. XXVIII, fig. 3). In hatching, the larva backs out of the egg. The eggs are easily broken, and it is probably due to this fragility and the fact that they are inserted far into the pores that the eggs of Lyctus beetles have apparently not been previously observed with absolute certainty of their identity.

SEASONAL HISTORY

Egg laying takes place principally during the middle of May. Recently hatched larvæ were first observed on June 1, 1915. The period of incubation is probably, at most, 10 days. The winter is passed in the larval stage. General pupation occurs about the first of April; the pupal cell (Pl. XXX) is excavated near the surface of the wood, and to this cell the larvæ retreat after cutting a transverse burrow nearly to the surface for the exit of the adults. General emergence of the adults takes place during May. Under normal conditions of the natural habitat of this species (in the Gulf and South Atlantic States) activity probably occurs earlier in the season.

There is apparently only one generation annually. But the combined work of the many larvæ of successive broods and generations burrowing through the wood results in the complete destruction of the interior and the conversion of the wood into fine powder—that is, "powder-posted" wood (Pl. XXIX, XXX, and XXXI).

CONCLUSIONS

Injury by "powder-post" beetles to unfinished seasoned wood products can be prevented by simply adapting a system of inspection, classification, and methods of disposal of stock to facts in the seasonal history of the insects, as has been recommended for many years by Hopkins (4, p. 6), Forest Entomologist. Such methods have been adopted by several large manufacturing companies with marked success.

In the case of finished wood products it may often be practicable to treat the wood with substances to prevent attack. Creosotes are effective preventives, but they stain the wood; hence, where they can not be used, in the light of the discovery of the place and manner of the laying of the eggs, any substance that will close the pores will prevent oviposition in wood not previously infested. In wood from which beetles have

[1] This is according to the law of orientation of Hallez (2).

emerged, however, eggs might be laid within the exit holes. Paraffin wax, varnish, or linseed oil effectively closes the pores of wood. Wood that has been seasoned less than 8 to 10 months will not be attacked by Lyctus beetles. In applying chemical preventives, only sapwood that has been seasoned for 8 to 10 months and longer should be treated. Judging from facts in the seasonal history of this species, preventives should be applied before March 1.

LITERATURE CITED

(1) DUGÈS, E.
1883. Métamorphoses du Lyctus planicollis Le Conte. *In* Ann. Soc. Ent. Belg., t. 27, p. 54–58, 1 pl.

(2) HALLEZ, Paul.
1886. Loi de l'orientation de l'embryon chez les insectes. *In* Compt. Rend. Acad. Sci. [Paris], t. 103, p. 606–608.

(3) HEEGER, Ernst.
1853. Beiträge zur Naturgeschichte der Insecten. *In* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Bd. 11, Heft 5, p. 927–942, 6 pl. Also reprinted.

(4) HOPKINS, A. D.
1910. Insects injurious to forest products. U. S. Dept. Agr. Bur. Ent. Circ. 128, 9 p.

(5) KRAUS, E. J., and HOPKINS, A. D.
1911. A revision of the powder-post beetles of the family Lyctidae of the United States and Europe. Pt. III. U. S. Dept. Agr. Bur. Ent. Tech. Ser. 20, p. 111–138.

(6) PIERCE, W. D., CUSHMAN, R. A., and HOOD, C. E.
1912. The insect enemies of the cotton boll weevil. U. S. Dept. Agr. Bur. Ent. Bul. 100, 99 p., 3 pl., 26 fig.

(7) XAMBEU, V.
1898. Mœurs et métamorphoses du Lyctus canaliculatus Fabricius. *In* Bul. Soc. Sci. Nat. Ouest France, t. 8, p. 69–72.

PLATE XXVIII

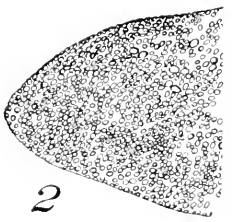
Lyctus planicollis:

Fig. 1.—Outline of the egg, showing strand attachment.

Fig. 2.—Greatly enlarged view of end of egg, showing granular appearance.

Fig. 3.—Larva within egg, ready to hatch. Drawn by Miss M. Carmody.

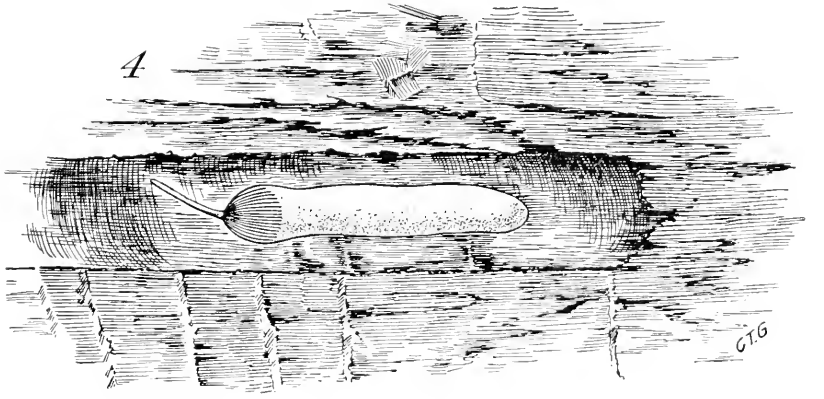
Fig. 4.—Sketch of egg in pore of wood on radial section of green-ash (*Fraxinus lanceolata*) ladder-rung stock, showing longitudinal striæ; pore opened to show egg. Drawn by C. T. Greene.



2

1

3



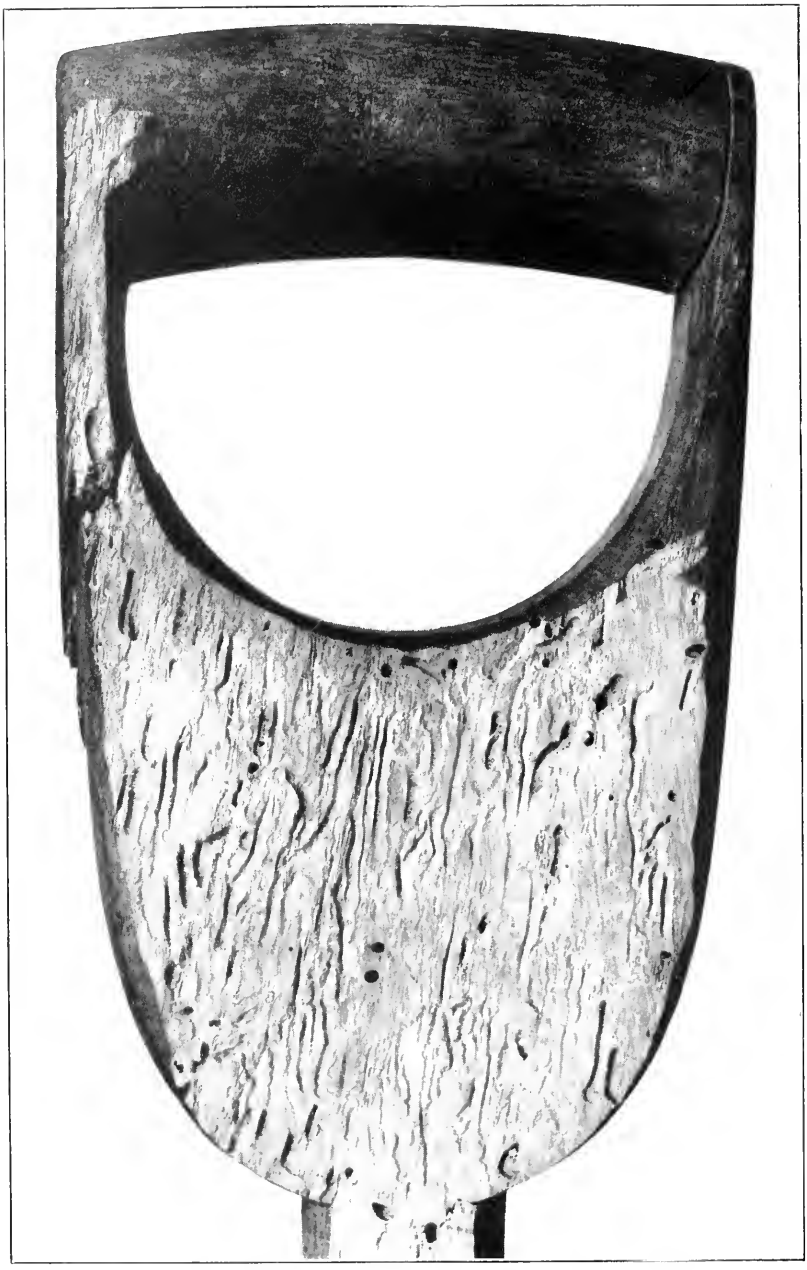


PLATE XXIX

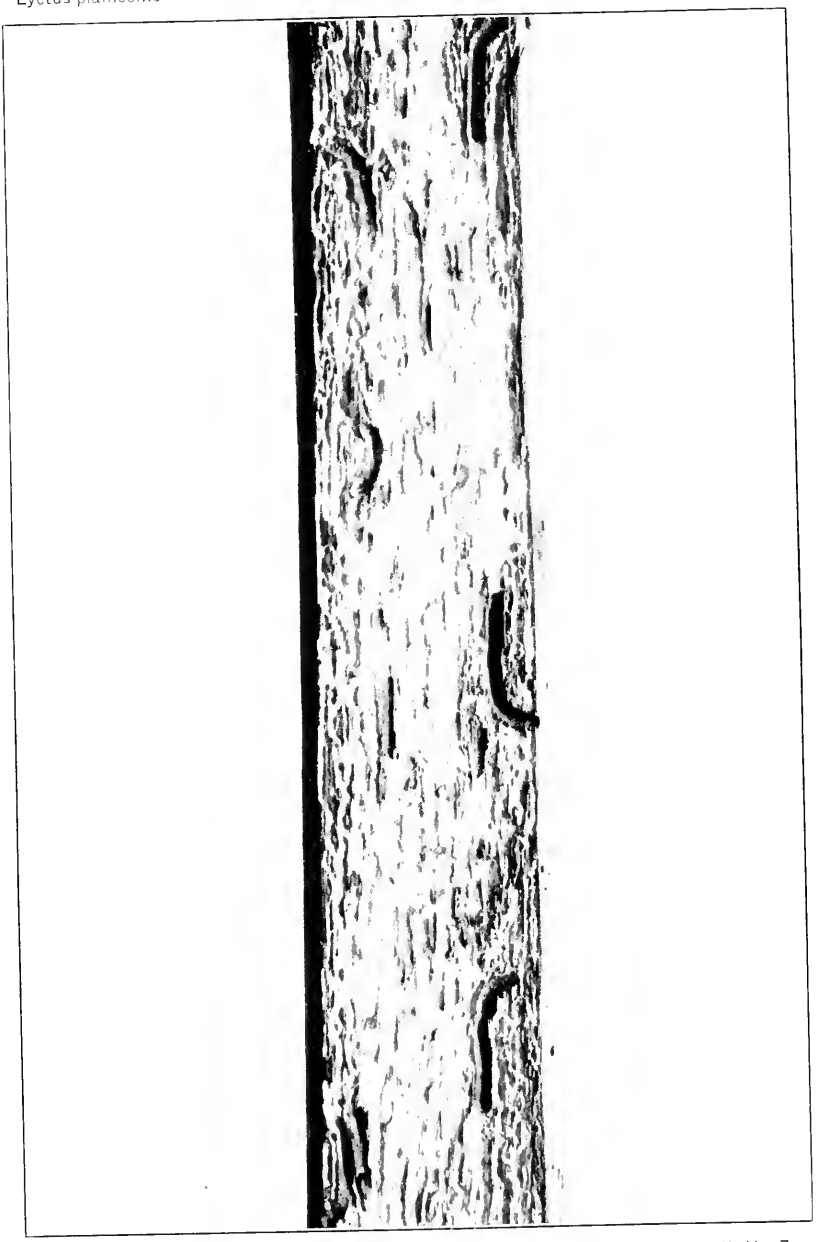
Lyctus planicollis:

Larval burrows in an ash shovel handle. Handle planed to show the work of the larvæ. Photographed by H. B. Kirk.

PLATE XXX

Lyctus planicollis:

Pupal cells in "powder-posted" white-ash shovel handle. Photographed by H. B. Kirk.



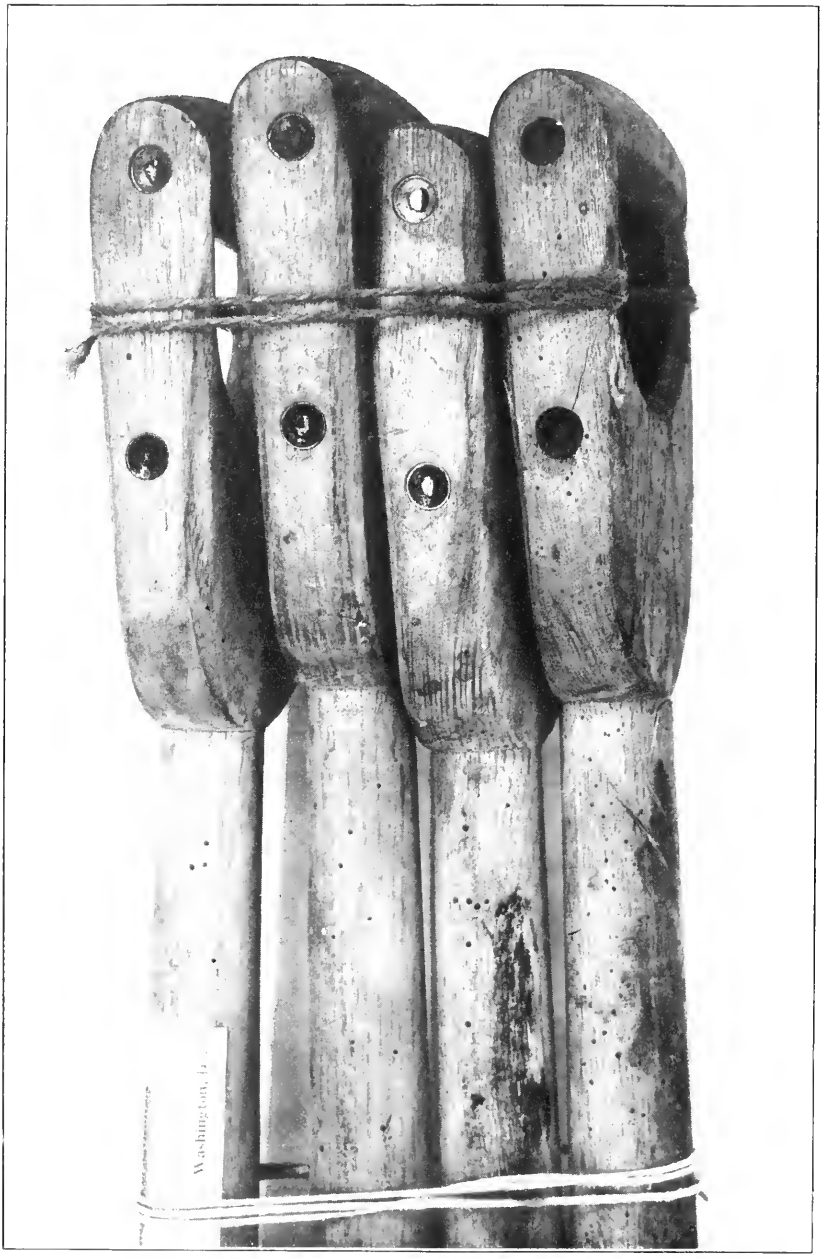


PLATE XXXI

Lyctus planicollis:

Exit holes of adults in ash shovel handles. Photographed by H. B. Kirk.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

10 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

Vol. VI MAY 22, 1916 No. 8

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., MAY 22, 1916 No. 8

HYPODERMA DEFORMANS, AN UNDESCRIBED NEEDLE FUNGUS OF THE WESTERN YELLOW PINE

By JAMES R. WEIR,
Forest Pathologist, Office of Investigations in Forest Pathology, Bureau of Plant Industry

INTRODUCTION

In the summer of 1913 the writer's attention was drawn to what appeared to be a very serious needle disease of the western yellow pine (*Pinus ponderosa* Laws.) in parts of Idaho, Washington, and Montana. That the disease has become more prevalent is shown by the receipt at the Laboratory of Forest Pathology at Missoula, Mont., of many collections of the fungus from localities where it was not before known to exist. These collections represent material from trees of all ages and show the youngest needles as badly diseased as the oldest ones. The first suspicion that the fungus might be of some economic importance arose through the discovery of a serious infection of young reproduction over a large area in the Whitman National Forest, Oregon. From the fact that the fungus causes a conspicuous hypertrophy by the extension of its mycelium into the tissues of the twigs and also through the destruction of the youngest needles, consequently causing in some localities much damage in the forest, it seems desirable to make known its characteristics.

TECHNICAL DESCRIPTION OF THE FUNGUS

Since the fungus does not agree with any known member of its genus, it is described as new.

Hypoderma deformans, n. sp.

Apothecia black, shiny, averaging 10 mm. in length and 1 mm. in breadth; may extend as a black line the entire length of the sheath side of the needle or be broken up into a series of shorter apothecia, usually arranged along the middle line of the needle, but may appear at either side and be very rarely confluent with the more medially arranged apothecia; opening with a longitudinal medial split. Asci fusiform (26) 26.1 to 43.5μ by 159.5 to 207.2μ (27.3 to 29.0μ by 171.5 to 186.4μ). Spores parallel or obliquely arranged in the asci, very generally slightly curved, uniform breadth, rod-shaped, ends blunt, 1-septate when mature, septum very conspicuous, cells often apparently separated, pale olive, almost hyalin, eight to an ascus (40) 6.2 to

9.7μ by 90.67 to 131.37μ (7.4 to 8.7μ by 108.9 to 117.6μ); paraphyses numerous, filamentous, swollen at the ends or recurved. Spermogonia intermixed averaging 5 mm. in length; spermatia elongated, straight, sometimes slightly curved, hyalin, continuous, averaging 1 by 8μ.

Type locality: Sumpter, Oreg., Whitman National Forest.

Habitat: Living needles of *Pinus ponderosa*.

Type material deposited in the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Washington, D. C., and in the collections for study in the Laboratory of Forest Pathology in the same office, at Missoula, Mont.

GENERAL BIOLOGY OF THE FUNGUS

The apothecia of the fungus are the most conspicuous of any of the group on pines in the West (fig. 1). From new infections of the previous year fully mature apothecia with well-developed spores (fig. 2) may be collected in early spring. From this time on the longitudinal split on the medial line of the apothecium is plainly visible, and may remain open or closed, depending on the humidity of the atmosphere.

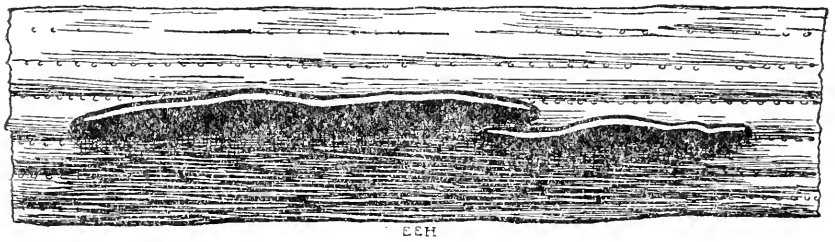


FIG. 1.—A side view of two apothecia of *Hypoderma deformans* on needles of *Pinus ponderosa*, showing the longitudinal medial split.

The splitting of the epidermis on the needle directly on the medial line of the apothecium is a characteristic shown by nearly all of the Hysteriaceae and in a few cases seems to be governed by a particular structure of the overlying layers of the apothecium. Thus, Von Tubeuf[1] points out that the pseudoparenchymous covering of the apothecium of *Lophodermium pinastri* (Schrad.) instead of being one continuous homogeneous tissue is made up of two parts which come together on the middle line of the fruiting body. The edges of the two parts interlock by a series of short papillæ. It is on the line of these papillæ, when the pressure within the apothecium becomes sufficient, that the epidermis of the needle ruptures. In *Hypoderma deformans* the rupture of the apothecium is apparently made easier by the coalescence of filamentous elements springing from the floor of the apothecium and meeting with the darker tissues of the apothecial covering above. Owing to a differentiation of the covering of the apothecium at the point of union a line of rupture is formed.

[1] Tubeuf, Carl von. Studien über die Schüttekrankheit der Kiefer. *In* Arb. Biol. Abt. Land- u. Forstw. K. Gsndhtsamt., Bd. 2, Heft 1, p. 22, 1901.

Pressure within the apothecium on approaching maturity, together with the elongation of the central elements, causes the rupture to occur on this line. After initiating the line of rupture, the filaments disappear and no sign of their presence exists when the spores are mature. In all material so far examined this mechanism is a constant characteristic. Where two apothecia are formed side by side, the filamentous structures are in marked contrast to the division line between the two apothecia as formed by the union of the darker colored elements of the apothecial covering. Von Tubeuf found in *Hypoderma strobicola* Tub. (*Lophodermium brachysporum* Rostr.) the same structure which he describes for *Lophodermium pinastri* (Schrad.), but no such structures were found in *H. deformans*.

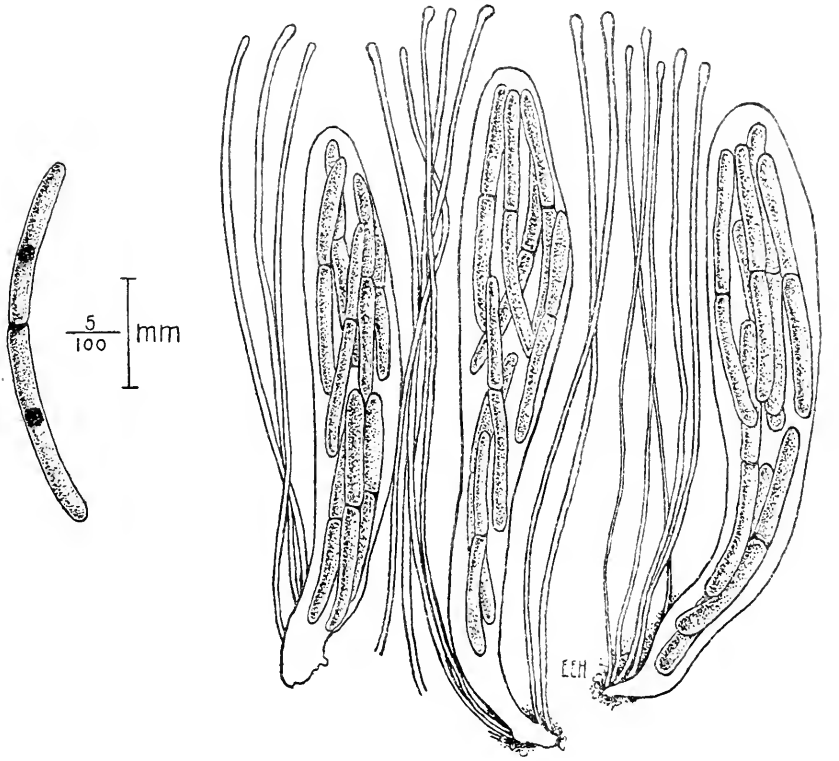


FIG. 2.—Asci, spores, and paraphyses of *Hypoderma deformans*.

Apothecia with mature spores (fig. 3) may be found at any season of the year. This is due to the fact that the spores do not ripen or are not all freed simultaneously when the split first appears in the apothecium. The process of spore liberation is observed to extend over a long period of time. A year may elapse before the apothecia have entirely liberated their spores. During periods of drought the medial slit in the apothecial covering remains closed, only opening on the return of abundant

moisture. The hygroscopic movements of the lips of the apothecium furnish the method by which the spores are forced or ejected from the asci. As Von Tubeuf [1] has pointed out in the case of *Lophodermium pinastri*, the spores are shot out from the mature asci under proper conditions of moisture. This fact is easily demonstrated by inclosing short pieces of previously moistened needles bearing mature apothecia in the cavities of plate-glass culture slides. A microscopic study of such preparations shows that the spores are shot out from the asci a distance of from 1 to 2 mm., showing as a plainly visible deposit on the floor and cover of the cavity. The depth of the cavity in the slides used was 2 mm.

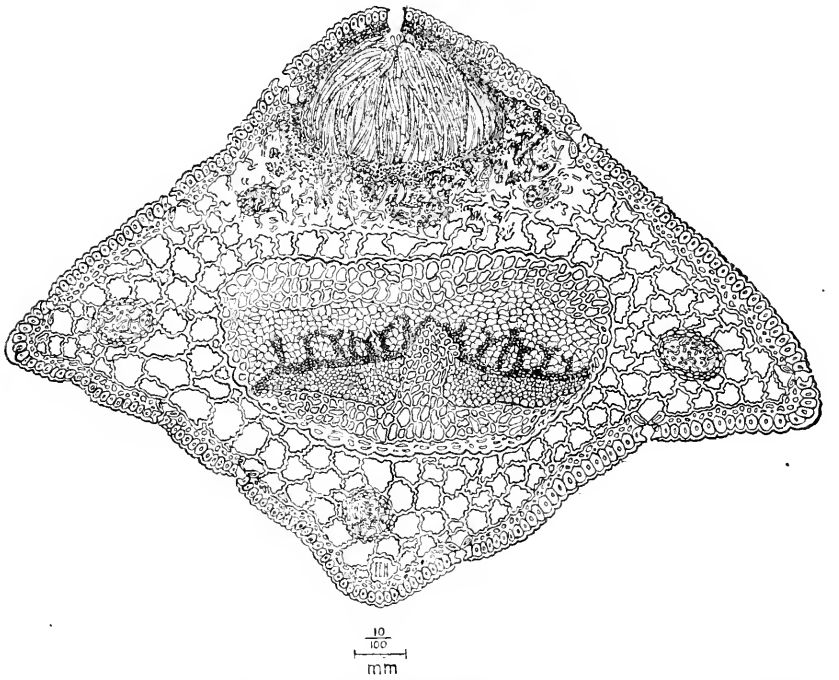


FIG. 3.—Cross section of an apothecium of *Hypoderma deformans* on a needle of *Pinus ponderosa*, showing mature asci with spores, the point of first rupture, and the tissues of the leaf most seriously affected by the mycelium of the fungus.

Occasionally an entire ascus was ejected and lay among the spores. In most cases, the asci remained attached and the spores were expelled through their terminal pores (fig. 4). Only the fully developed spores were cast out of the apothecia. After the material had remained in the slides a day and a half, during which time the spores were being ejected, the cover glass of a slide was removed and the material allowed to dry by exposure to the air of the laboratory for 30 days. The material was washed and replaced in the cavity in the slide. Within three hours spores from the same apothecia were expelled in considerable numbers but not so profusely as before. The process was repeated with shorter

[1] Tubeuf, Carl von. Op. cit., p. 24–25.

periods of drying till on the fourth trial no spores were liberated. An examination of the apothecia showed the asci to be entirely empty. This experiment not only demonstrates that the fungus has the ability to resist protracted periods of dryness but that the period of spore liberation may be much protracted, depending upon the atmospheric humidity. During wet weather apothecia expel their spores in visible quantity when a sharp blow is given the branch bearing infected needles.

Considering the long periods of drought in most yellow-pine regions, it is safe to assume that an apothecium ripening in early spring may first become emptied of its spores during the ensuing winter or even later. This is important for the propagation of the fungus, since new infections are possible from the time the first needles of the season appear till the close of the growing season.

In order to determine the viability of the spores expelled from apothecia after long dryness, a 2 per cent sugar solution was introduced into the cavity of one of the slides containing apothecia which had lain dry in the laboratory for two months and the slide placed in the thermostat at 35° C. On the fourth day spores germinated readily. The germ tubes appeared more frequently from the ends of the spores. A slight addition of an extract of pine needles to the sugar solution promoted germination.

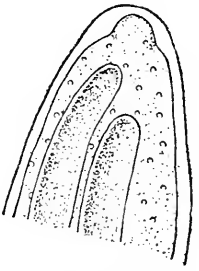


FIG. 4.—The upper portion of a young ascus of *Hypoderma deformans*, showing the formation of the pore at the tip through which the spores are expelled.

It was noticed that in collections of the fungus made shortly after warm summer rains the asci are frequently empty as compared with asci of mature apothecia collected in the colder spring months. This, it seems, may not be entirely due to a longer period of spore liberation but also to the higher temperature of the summer months. Von Tubeuf found that increasing temperature promoted spore liberation in *Lophodermium pinastri* and it is found to be true in experiments with the yellow-pine fungus. During the winter, moistened apothecia from dry material were mounted in two culture slides; one was placed outside the laboratory during a period when the thermometer registered about 40° F. and the other was kept in the laboratory air of about 80° F. At the end of four hours a microscopical examination showed that a large number of spores had been ejected from the apothecia in the slide kept in the laboratory but none from the other slide. When the slide from the outside was allowed to stand for a while in the warm air of the laboratory, spores were liberated in quantity.

Although spores from various needle fungi are undoubtedly more readily liberated during warm rains of the summer months, the frequent drying of the foliage of the trees is probably not favorable for infection. It is frequently observed, and as often reported, that needle fungi become more active during the cool, protracted rainy periods of early spring and late fall. No extensive data are at hand regarding the resistance of

expelled spores to drought and direct light; still, the fact that dry herbarium material a year old was found to furnish viable spores shows that spores may exhibit considerable resistance to dry air when free from the apothecium.

PARASITISM OF HYPODERMA DEFORMANS

An attempt to grow the fungus on culture media failed. The spores in every case germinated and in some cases produced an abundant white mycelium, but in the course of six months, after frequent transfers, the mycelium turned a light yellow and died. A somewhat better result was obtained by adding to the culture medium a strong extract made from yellow-pine needles in water, but at the end of eight months the mycelium died.

A quantity of needles bearing apothecia with mature spores were collected in the spring of 1914 near Missoula, Mont., and taken to the field station in the Priest River Valley, Idaho, for experiments on parasitism. The fungus has not been found in this region. The needles were thoroughly washed in distilled water and the apothecia allowed to expel their spores in small sterilized flasks. Needles and spores were shaken up in water to which a 1 per cent sugar solution was added. The mixture was allowed to stand one day and then thoroughly sprayed over four 3-year-old yellow-pine seedlings having young tender shoots with needles. The inoculated seedlings were immediately inclosed in tough, transparent oiled paper bags and protected from injury. A second experiment was initiated by binding infected needles on healthy 3-year-old seedlings. In the part of the Priest River Valley where these experiments were performed the yellow pine is not common, being only sparingly represented in a mixture of white pine, grand fir, spruce, hemlock, and Douglas fir. The experiments were made on May 20. In September the last-formed needles of the inoculated seedlings were turning reddish brown in spots, mostly at the tips. In the following spring, May to June, the needles which showed infection in the fall and which had become wholly brown developed the characteristic long, shiny black apothecia with mature spores (Pl. XXXII, fig. 1). Only the needles formed during the previous year were infected. Four control plants, also covered with bags, were entirely free from the disease. The needles of the seedlings on which infected needles were bound showed a much more general infection of the last-formed needles than those by the former described method. In these experiments every needle produced in 1914 was infected. Those of previous years remained healthy. This indicates that old needles are not attacked and that the young needles may remain attacked indefinitely after infection. All the infected needles did not produce mature apothecia. Those merely turning brown were filled with the mycelium of the fungus. The experiment at this point was discontinued. In all probability, given time enough, the brown-infected needles would have produced apothecia.

It has been noticed repeatedly in nature that there is great irregularity in the time between the first browning of the needles at their tips or at other points along the needles and the appearance of the mature apothecia. In a few cases the cycle of development from the first appearance of the brown color at the tips of the needles to mature apothecia has been observed to take place within the same calendar year, or from April and May to November. More often infected needles first showed mature spores in the spring of the following year. It was observed in a few cases that the needles may lie on the ground through the following winter before the apothecia rupture. Brown needles collected in August from infected trees and placed in damp moss in the field in a number of cases developed apothecia before January, maturing in May and June. The apothecia, as previously indicated, may contain asci in various stages of development, so that mature spores are being produced throughout the year. Investigation has shown, however, the greatest number of spores are expelled during the spring rainy season, May and early June, coinciding with the greatest vegetative period of the host. In no instance, either in the field or in artificial inoculation, were the infected needles of young trees or seedlings not previously attacked by the fungus killed before they had attained their normal size. In September or October, such needles will have assumed a more or less uniform reddish brown color. Mostly remaining upon the tree, they may first produce the signs of the apothecia during the late fall and mature the spores in the following spring. At the time the foregoing experiments were in progress small bundles of infected needles bearing fertile apothecia were bound with similar quantities of needles which had died from a normal cause. These were placed in moss during May, 1914. On examination in May of the following year the needles which had died from a normal cause showed no signs of the fungus; nor have they done so since that date. This apparently demonstrates the inability of the fungus to act as a saprophyte.

The foregoing observations and experiments apparently prove the parasitism of the fungus. This is further substantiated by the observed evidences that young seedlings in the field succumb to the ravages of the fungus. Furthermore, it is indicated that the period of greatest infection is during the growing season and only the needles of the season are to any extent susceptible to attack.

The fungus has not yet appeared in the forest nursery, but it may be regarded as a possible nursery disease.

PATHOLOGICAL EFFECTS OF THE FUNGUS ON THE BRANCHES OF THE HOST

A very peculiar and at the same time interesting phenomenon caused by the growth of the mycelium of the fungus in the shoot is the formation of spherical-shaped witches'-brooms on trees mostly past the seedling stage. These (Pl. XXXII, fig. 2) brooms in old trees often assume large proportions. A single witches' broom may weigh as high

as 100 pounds and measure 5 or 6 feet in diameter. The branch supporting it will hang vertically, the broom swaying in the wind like a great bag (Pl. XXXII, fig. 3). The average size of the brooms is about 2 feet in diameter. Although a few isolated cases had been noted on the seeming association of this needle fungus with these compact brooms, it was not until the field season of 1913 that this association was found to be of common occurrence. This was all the more interesting from the fact that the cause of these formations has been a standing question with all who have seen them. In some cases they have been attributed to the yellow-pine mistletoe, *Razoumofskya campylopoda* (Engelm.) Piper, an error, however, not likely to be made by anyone familiar with the type of broom caused by this mistletoe.

The distribution of the brooms is quite general through the range of the yellow pine in the Northwest. They are particularly abundant in the vicinity of the great lakes of Idaho and in the dry valleys of southern and western Montana. Climatic variation does not seem to influence their distribution.

In order to determine the cause and nature of the formation of these brooms and the relation, if any, between them and the fungus common on their needles, the subject has been under investigation in the field and laboratory. A number of interesting observations have been recorded.

The disease caused by *H. deformans* primarily affects the needles. In young pines the disease occurs quite generally at first, unaccompanied by any kind of hypertrophy of the shoots. Later the repeated destruction of the last-formed and older needles initiates a swelling of that portion of the branch. Sometimes the entire shoot succumbs to the attack in seedlings of tender years, especially the weaker individuals, caused, no doubt, by the rapid drying out of the shoot. In growths of 7 to 10 years the fungus confined itself to the needles of the season, with the result that on the infection of these a second crop sometimes appears about the terminal bud, which may or may not become infected but may remain in a stunted, deformed condition. They help, however, to maintain the shoot in a living condition. In a far greater measure than in any other member of the order the mycelium of *H. deformans* penetrates the leaf sheath and eventually perennates in the tissues of the shoot, causing a marked enlargement of the parts infected. The fungus, however, fruits only on the needles.

An additional result of the infection of the terminal shoots and the continued production of food materials by the older, uninfected needles is the stimulation of all lateral and adventitious buds either between the primary terminal buds or at the last two or three nodes. Eventually, the food materials are more and more diverted from the main shoot, resulting in a gnarled and curved bunch of short branches. Young trees

4 to 8 years old when uniformly infected are frequently observed with the terminal portions of every principal branch in the process of "brooming." The fact that the fungus sometimes occurs without the least sign of a hypertrophy of the branch does not indicate that it is not capable of producing such physiological and morphological changes. The fact remains that on all young growth almost always the twigs bearing the infected needles are abnormally swollen or branched. The fungus has not been found by the writer on mistletoe brooms or on any form of broom caused by insect or other animal injury. On large and mature trees *H. deformans* very rarely occurs on any part of the tree except the needles of these brooms. These abnormalities are scattered promiscuously over the tree, but principally on the lower branches. This indicates the nature of an infection. The more recent infections on old trees are usually distributed or isolated on particular branches. Serious injury seldom results from the growth of the brooms on more mature growth. Very rarely may the brooms become so heavy as to split-off the supporting branch.

As the result of an examination of the witches' brooms on yellow pine in the Bitter Root and Missoula River valleys, Montana, and the Coeur d'Alene region of Idaho, with respect to the presence of *H. deformans* on the brooms and the number, position, and distribution of the brooms on the tree, the following data were obtained:

On 107 trees examined, the average number of witches' brooms per tree was 3.2. These brooms generally appeared on the lower part of the crown on the side facing the prevailing winds. The average number of brooms per tree bearing needles showing apothecia of *H. deformans* was also 3.2.

These figures support the view that the peculiar brooms so common on yellow pine are the result of fungus infection and that the fungus responsible is *H. deformans*.

In the parts of northern Washington, Montana, and Idaho so far visited, *H. deformans* has not been found to attack the yellow-pine reproduction in as great a degree as in regions farther to the west and south. This is probably due to a greater mixture of species. The fungus is not able to spread with the same rapidity as in the more typical yellow-pine stands. The infected young growth usually continues alive indefinitely, and deformed branches appear, eventually resulting in an entire retardation of growth, and finally die. This process may require several seasons, but the infected pines never attain a very large size. Such deformed trees usually are attacked by bark beetles, such attacks hastening their decline.

In parts of Oregon in the yellow-pine belt the fungus was found to be very destructive. During an investigation of the larch mistletoe (*Razoumofskya laricis* Piper) in the vicinity of Sumpter, Oreg., the

yellow-pine reproduction, especially on south slopes under mature cover, was observed to be turning brown and in many cases dying. On examination the needles of these seedlings showed that they were infected with *H. deformans*. This is a grazing region, and the forest has been continuously grazed by large bands of sheep for many years. The stems of the young pines in numerous cases bore near their bases one or more wounds of a shape and nature indicating that they were produced by the treading of grazing animals. Since little information is at hand on the effects on forest production of wounding by grazing animals, it seemed worth while to make a detailed study of the case so far as time would allow, with the double object of determining which injury—viz, the needle fungus or the wounding—was responsible for the sickly condition of the young pines. It must be remembered, however, that the seedlings were growing under the canopy of a mature yellow-pine stand; consequently they were not growing rapidly in height. Four one-tenth-acre plots were laid off on representative south slope sites and every seedling on the plots carefully pulled up and bound in bundles. These bundles were sent to the laboratory and afterwards carefully diagnosed. The normal condition of root system and crown and general vigor of seedlings were judged from a knowledge of normal young pines of the same age, free from disease and wounds, growing in the same regions and under the same slope conditions. The results of this study were embodied in a preliminary table from which Table I was condensed as being more readily undestandable.

TABLE I.—*Number of seedlings on 4 one-tenth-acre plots, average age and height, condition of infection with Hypoderma deformans, present condition of wounding and root system, south-slope type*

Condition of seedlings.	Number of seedlings on 4 one-tenth-acre plots.	Average age.	Average height.	Vigor and general appearance of seedlings.
		Years.	*Feet.*	
Seedlings neither wounded nor infected.	40	10. 7	2. 8	Healthy green color, vigorous, normal, well developed root system.
Seedlings infected with *Hypoderma deformans* but not wounded.	67	13. 0	1. 3	Few green needles, most all badly infected and either dead or dying; twigs twisted or broomed; poorly developed root system; general picture of a starving condition.
Seedlings both wounded and infected.	127	12. 1	1. 4	Do.
Seedlings wounded but not infected.	49	12. 8	2. 14	Wounds mostly healed. Time required to heal, 1 to 4 years. Seedlings normal. Wounding apparently not affecting growth. Root system normal.

DISTRIBUTION OF HYPODERMA DEFORMANS

The disease of yellow-pine needles caused by *H. deformans* is widely distributed throughout the northwestern part of the United States and western Canada. Its distribution in other parts of the West is not known, although the fungus has undoubtedly been collected

by other observers.[1] The writer has observed and collected *H. deformans* in the National Forests of the Northwest as follows: Sioux, Helena, Deerlodge, Jefferson, Missoula, Coeur d'Alene, St. Joe, Clearwater, Selway, Bitterroot, Pend Oreille, Kaniksu, Nez Perce, Lolo, Cabinet, Flathead, Kootenai, and Whitman. The late J. F. Pernot, forest examiner, supplied specimens from the Deschutes, Wallowa, Malheur, Crater, Colville, and Wenatchee National Forests. Along the Thompson River in British Columbia the fungus was occasionally found by the writer in the summer of 1913.

CONCLUSIONS AND RECOMMENDATIONS

A very conspicuous disease on yellow-pine needles in many parts of the Northwest, the cause of which has for several seasons remained unknown, is found to be caused by a fungus which is described as a new species under the name "*Hypoderma deformans*."

H. deformans is a true parasite and attacks the foliage of all age classes; and in some of the more exposed sites of the typical yellow-pine belt of Montana, Oregon, Washington, and Idaho, young seedlings at first suffer great suppression and are finally killed.

The first sign of infection of the needles is usually a slight browning of the tips; or in the regions of heavy infection the entire needle may gradually assume a straw-yellow color, deepening to a brown on the first appearance of the apothecia.

Because of the destruction of the youngest needles and the penetration of the mycelium of the fungus in the tissues of the stems of the host, the terminal shoots do not attain their proper development, but become stunted and deformed, eventually producing a witches' broom. These witches' brooms on young yellow-pine saplings or older trees are often very conspicuous and often occur in such numbers as to make either an individual tree or an entire stand look very ragged and unsightly.

Up to the present time the disease has not been found in the forest nursery, but it may be regarded as a possible nursery disease. Since the vegetative mycelium of the fungus may hibernate in the shoots of seedlings after the infected needles have fallen, the fungus may make its appearance in the forest nursery and may be unknowingly transferred to the planting areas.

The presence of the fungus on mature forest trees is very readily recognized by the foliage browning up in patches or by the formation of brooms. Since the fungus does not affect the merchantability of the tree, except by influencing the increment in cases of very severe infection, all trees of the regulation diameter classes should be marked for

[1] Meinecke describes a very destructive needle fungus, under the name "Hypoderma," on yellow and Jeffrey pines, which apparently is the same fungus as the one described in these pages. (Meinecke, E. P. M. Forest Tree Diseases Common in California and Nevada, p. 54. Washington, 1914. Pub. by U. S. Dept. Agr. Forest Serv.)

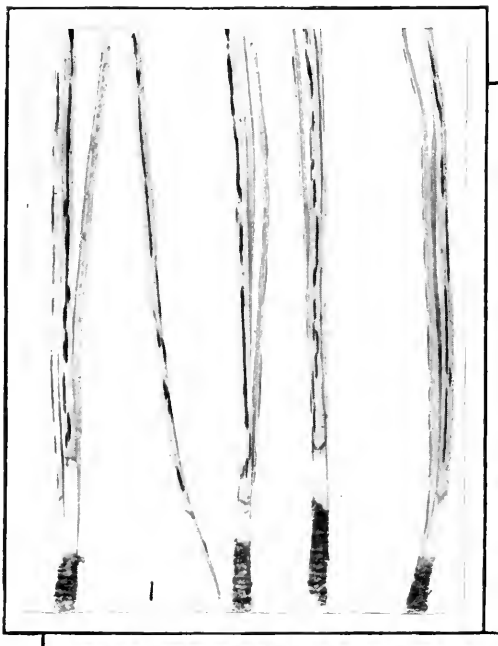
cutting. The brooms never produce cones and the normal parts of the supporting branch are usually sterile. The branches bearing patches of infected needles or brooms should be piled and burned as soon as possible. This may be done in the course of the regular brush-piling operations. If young trees below the regulation cutting diameter are so badly "broomed" that in the opinion of the forest officer the increment of the tree will be seriously impaired, and whenever the cost is not prohibitive, such trees should be lopped and immediately burned. The chief reason for such procedure is to protect the reproduction from infection, thus insuring a healthier forest in the future.

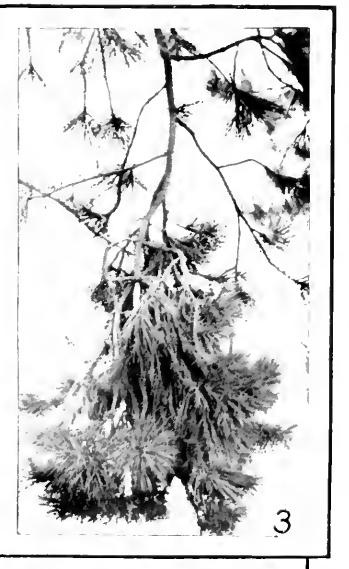
PLATE XXXII

Fig. 1.—Needles of *Pinus ponderosa* infected with *Hypoderma deformans*, showing the apothecia. Natural size.

Fig. 2.—Branches of *Pinus ponderosa* deformed and broomed by *Hypoderma deformans*.

Fig. 3.—A branch of *Pinus ponderosa*, showing how it will hang vertically when supporting a large broom caused by *Hypoderma deformans*.





ORNIX GEMINATELLA, THE UNSPOTTED TENTIFORM LEAF MINER OF APPLE

By L. HASEMAN,[1]
Entomologist, Missouri Agricultural Experiment Station

INTRODUCTION

The small, unspotted tentiform leaf miner (*Ornix geminatella* Pack.) has been extremely abundant in Missouri in recent years and has attracted the attention of fruit growers throughout the State. It has confined itself largely to bearing apple (*Malus sylvestris*) orchards, though considerable injury has been done to apple foliage in nurseries. Fortunately, it is most abundant in the late summer and early fall, so that its work is of less importance to the trees. As with many insect pests, it seems to run in cycles. It was most abundant during the summers of 1911 and 1912, reaching a climax in 1912. Since 1912 it has attracted little attention.

It confines its work to the leaves and spends most of its larval life inside the leaf as a true miner. The caterpillar therefore is small, though the characteristic elevated, or tentiform, dead patches which it produces on the leaves are quite noticeable. In some cases as many as 15 mines have been found on a single large apple leaf (Pl. XXXIII, fig. 14, 15). The pest was so abundant and so widely distributed throughout the State that a careful study of its life history, habits, and control was undertaken.

HISTORY OF THE PEST

The moth was first described and figured by Packard (7, p. 353)[2] in 1869 as *Lithocolletes geminatella*. The description and figures are incomplete and not entirely accurate, owing perhaps to incomplete observations. Since its first discovery it has been collected by various workers and was redescribed by Chambers (2) as *L. prunivorella*. Other closely related micros have been mistaken for it, and some careful observers have given very inaccurate descriptions of its work and habits.

DISTRIBUTION OF THE LEAF MINER

Packard reported it as being abundant in New England on pear and apple; Lowe (6) reported it as being very abundant on apple in New

[1] The writer wishes to acknowledge his indebtedness to the late Miss Mary E. Murtfeldt, of Kirkwood, Mo., to Miss Annette F. Braun, of Cincinnati, Ohio, and to Mr. August Busch, of the Smithsonian Institution, Washington, D. C., for assisting with the naming of the leaf miner; and to Dr. L. O. Howard, Chief of the Bureau of Entomology, and to Mr. A. A. Girault, of the same Bureau, for the determination of the parasites. He is also especially indebted to Prof. C. R. Crosby, of Cornell University, for helpful suggestions and for assistance in naming the leaf miner and the parasites.

[2] Reference is made by number to "Literature cited," p. 295.

York, and Brunn (1) reported it from Ithaca, N. Y. Forbes (4, p. 57) reported it from Illinois, New York, Colorado, Kentucky, Michigan, and Massachusetts; and Jarvis (5, p. 49) reported it as being common in Connecticut. Dietz (3) reported it from the Middle and Northern States of the Atlantic slope, though he confused species. In a recent attempt to determine its present distribution the writer has been able to get definite records from but one additional State, Ohio. It is probable that it is found from the Atlantic States to Colorado, but being so small and inconspicuous, except when abundant, fruit growers and entomologists have overlooked the insect and its work.

LIFE HISTORY OF THE MINER

The writer has not been able to find any report of the complete life history of the pest. Such records as are available deal with the insect and its development and work in the summer or more often for a short period in the late fall. In some cases very careful data have been recorded, but many of the records and descriptions are decidedly at fault. The following records for the insect in Missouri have been collected since the summer of 1911 and include new data on the life history, development, and habits of the pest.

EGG

The egg is extremely small, slightly oblong, varying from 0.254 to 0.4 mm. in length and from 0.18 to 0.29 mm. in breadth, only slightly elevated and firmly cemented invariably to the lower surface of the leaf. (Pl. XXXIII, fig. 3.) It is so small that it can scarcely be detected with a hand lens, and the writer has failed to find the unhatched eggs on foliage, though many have been collected and studied soon after hatching, when the young caterpillar had just begun to start its mine. The adults have refused to lay eggs in captivity in small vials; therefore, these records are for the freshly hatched eggs.

THE LARVA

On hatching, the larva is footless and resembles a microscopic flat-headed borer. It always seems to break through the part of the shell which is cemented to the leaf and enters the tissue of the leaf at once. The freshly hatched caterpillar is less than a millimeter in length. It grows rapidly and when mature is about 6 mm. in length. In its development it passes through four distinct larval stages. There is considerable variation in size, but the following measurements are the average of many specimens.

In the first stage the caterpillar is pale, with a slight yellowish tinge to the head. The head and thorax are enlarged and it is footless. It molts when it is yet less than 2 mm. in length, and the head capsule is about 0.18 mm. in breadth. (Pl. XXXIII, fig. 4.)

In the second stage the body is pale, the head becomes brownish, a black blotch begins to appear on the first thoracic segment, legs are still absent, the head capsule is about 0.27 mm. broad, and the caterpillar is about 2.2 mm. long. (Pl. XXXIII, fig. 5, 6.)

In the third stage the body is at first pale, but darkens with age; the thoracic and abdominal legs appear; the thoracic blotch breaks up into four irregular spots; the head becomes darker and is about 0.35 mm. in breadth, while the caterpillar is about 4.5 mm. in length. (Pl. XXXIII, fig. 7.)

In the fourth stage the caterpillar is about 6 mm. long and the head capsule is 0.49 mm. broad; the body takes on an olive-gray color, sharply contrasting with the conspicuous white tubercles; the head becomes darker, and along its hind margin appears a row of four small black spots which parallel the similar row of larger spots on the first thoracic segment. (Pl. XXXIII, fig. 8, 9.)

THE MINE

While the caterpillar is changing from a pale, flat, footless, microscopic caterpillar to a conspicuously marked, cylindrical, active one, its mine also undergoes distinct changes. At first the mine is serpentine in form; but after it is from 4 to 8 mm. in length and is usually curved upon itself, the caterpillar begins to transform it into a blotch mine. (Pl. XXXIII, fig. 13.) The blotch mine begins by the third day, and about that time the caterpillar changes to the second stage. At first the blotch appears only on the lower side of the leaf. The lower layer of the leaf is separated from the upper by the flat caterpillar, and soon the severed lower layer dies and turns brown. The mine remains in the blotch stage about four or five days, and during that time the caterpillar changes to the third stage. When complete, the blotch is from 1 to 2 cm. long and usually occupies all the space between two of the main lateral veins of a leaf. On preparing to produce the tentiform mine, the caterpillar spins silk threads on the floor of the mine, which causes the lower dead layer of the leaf to become folded lengthwise of the mine. These threads, with others spun later under the roof of the mine, cause the upward projection of the mine. Just about this time the caterpillar changes to the fourth stage and begins to feed on the chlorophyll cells, and this in time gives the unspotted effect when a clear net work of veins appears. During the latter part of June it was found that in from a week to 10 days after the young caterpillar begins to feed, the mine is changed from the serpentine through the blotch to the tentiform type. The majority of the feeding and growth occurs in the third and fourth stages, and after the tentiform mine is made it requires from four to seven days to eat out all the chlorophyll cells and give it the completed, unspotted, tentiform appearance. The larval life in the mine is therefore about two weeks. The caterpillar leaves

the mine through a small hole in the floor of the mine and after crawling about for a varying length of time prepares to make a cocoon in which to pupate.

COCOON

The cocoon is almost invariably made on the upper surface along the edge of the leaf or at its very tip. On preparing to make the cocoon the caterpillar first rasps off and eats a patch of the surface layer of cells along the edge of the leaf, about 4 mm. wide and twice that in length. This causes a withering of the tissue and a slight folding over of the edge of the leaf. Then begins the work of spinning silk. First a loose layer of silk threads is spun from a line about 2 mm. from the edge of the leaf to the inner edge of the patch rasped off. Then follows a second layer of threads which are drawn very tight as they are placed. The anterior two-thirds of the body of the caterpillar enters into this work with great energy and force. The caterpillar's silk press must be a strong one. This layer only slightly draws up the edge of the leaf, but after transversed bands are used to tie the tight threads in bundles the edge of the leaf is perceptibly folded. At this time a second layer of foundation threads are spun underneath and then the work of drawing tight threads is continued along one end of the future cocoon. In half an hour the leaf edge is half drawn over and the hardest part of the work is completed. After the edge is tied down tightly the inclosed space is thoroughly lined with snow-white silk, so that a very dense semicircular cocoon 8 mm. long is formed. (Pl. XXXIII, fig. 12.)

PUPA

The mature caterpillar pupates soon after the cocoon is completed. The pupa is about 4 mm. long, exclusive of the antennal sheaths which project fully a millimeter beyond the tip of the body (Pl. XXXIII, fig. 10, 11). The pupa darkens with age, becoming dark brown on the dorsum and yellowish brown on the venter. The leg, wing, and antennal sheaths are all distinct. The pupal period varies from a few days to a week in midsummer.

MOTH

The newly-emerged adult on assuming its full splendor is truly a beautiful creature when viewed through a microscope. When left undisturbed it will stand perfectly still for hours, with the head elevated and the tip of the wings and abdomen lightly touching the surface on which it rests (Pl. XXXIII, fig. 2). This is its characteristic pose, and it holds it so perfectly that prolonged exposures for enlargements can safely be made. While in this pose the light flashes from every properly arranged scale as from polished metal, and one who is only familiar with the appearance of museum specimens can hardly appreciate the peacock-like splendor of this seemingly proud little creature.

Brunn's (1) description of the adult is very good. To the unaided eye the moth is slate-gray with a slight tinge of brown, being lighter in ruffled specimens. The ventral surface of the body is lighter in color. The markings on the front two pairs of legs are similar. The tarsal segments are white, tipped with black; the tibia and femur vary from dark brown to black with lighter patches; the coxæ are mottled with white and dark scales. The tarsal segments of the hind legs are brownish with white basal bands, while the tibia, femur, and coxa are much lighter, being nearly the same color as the lower surface of the abdomen. The palpi are prominent and banded with white and dark scales. The brownish proboscis is unusually long, reaching to beyond the base of the abdomen which, though it has not been observed to do so, would lead one to conclude that the moth feeds. The antennæ are brownish in color and distinctly annulate with whitish. In life they are closely pressed along the sides of the body and reach to beyond the tip of the abdomen and wings.

The surface of the forewings is beautifully mottled with light and dark scales. The light scales are arranged in eight or nine more or less distinct transverse bands. In museum specimens it is difficult to distinguish these bands. Near the tip of the forewings in fresh specimens, is a distinct black patch of scales bordered without by three alternating, narrow, white and black curving bands, giving to the tip of the wings a distinct peacock spot. On the hinder margin of the front wings the black and white scales forming the terminal peacock spot give way to long, light-colored hair. This border of delicate hair ceases near the middle of the hinder margin of the wing. The hind wings are slender and armed on the hinder margin with a broad band of delicate light-colored hair. This band becomes narrower toward the tip of the wing. The costal band is scarcely as broad as the wing (Pl. XXXIII, fig. 1).

The moth has a wing expanse of from 7 to 9 mm. and is approximately 5 mm. long when at rest with the wings folded.

NUMBER OF BROODS

This species winters in the pupa stage in a carefully prepared cocoon protected by the folded-over edge of a leaf. In the spring the adults are abundant by the first week in May. By the middle of May the typical tentiform mines begin to appear, and the adults of the first spring brood begin to emerge by the last of May. The life cycle is completed in from four to five weeks. The broods overlap, but beginning with May a fairly well-defined brood can be made out for each month until November. The larvæ of the October brood pupate and live through the winter on fallen leaves. After the moths emerge a considerable period of time elapses before the mines begin to appear. This is undoubtedly due to the fact that the moth, with its well-developed proboscis, feeds for a time before ovipositing.

FOOD PLANTS OF THE LEAF MINER

This leaf miner is primarily a pest of the foliage of the apple. There is where it abounds. However, the small caterpillars have been found developing in considerable numbers in the leaves of the crab-apple (*Malus* sp.), and to a less extent in the leaves of the haw (*Crataegus* spp.), plum (*Prunus* spp.), cherry (*Prunus* spp.), and pear (*Pyrus* spp.). In the case of the last four trees only an occasional mine has been observed. Chambers (2) and others have also reared it from mines in the leaves of the wild cherry (*Prunus* spp.).

CONTROL OF THE LEAF MINER

While this miner may develop in such numbers that from 90 to 95 per cent of all leaves on apple trees may contain from 1 to 10 or 15 mines, it must be said that it is not an especially alarming pest of the orchard (Pl. XXXIII, fig. 14, 15). The pest increases in abundance as the summer and fall advance, so that by September or October much of the foliage may be consumed, but by that time the tree has about completed its growth and matured its crop. However, when conditions are favorable and the pest is abundant, steps should be taken to prevent it from reappearing in injurious numbers the next season.

Since the caterpillar enters the leaf immediately on hatching and remains in the mine until mature and ready to spin its cocoon for pupating, arsenical and contact sprays are of no value. Applications of sprays have given the writer absolutely no results. From the general nature of the pest and its habits, there seems to be no feasible means of controlling it during the growing season. Since it passes the winter as the pupa in cocoons on fallen leaves, it can be effectively controlled by destroying the leaves early in the spring. The most practical method of destroying the pupæ on the leaves is to use a disk for shallow cultivation before the first of March so as to work under the leaves before the moths begin to emerge. Summer cultivation will not help, since the pest is not found on the ground at that time. In a small home orchard the leaves can be raked together and burned or piled and used for leaf mold. If they are not burned, they should be covered with enough soil or stable manure to hasten the decay of the leaves and prevent the moths from emerging in the spring.

PARASITES OF THE TENTIFORM LEAF MINER

It would seem that a caterpillar of this type, which lives protected inside the leaf from the time it hatches from the egg until it is ready to pupate, would be as well protected from natural enemies as from artificial treatment given by man. This does not prove to be the case, however, for the pest is heavily parasitized. It resembles other insect pests which are subject to the attacks of parasites in that under favorable

conditions it increases rapidly and then when the parasites get the upper hand it suddenly disappears. In the summer of 1912 it reached a climax as regards abundance. During the fall the parasites increased in such numbers that but few of the caterpillars escaped to pupate and pass the winter. The check, owing to the beneficial work of the parasites, was complete, for the miner has not attracted attention since 1912.

As the investigation of the miner progressed, it was observed that many of the mines went no farther than the blotch stage, while others arrived at the tentiform stage; but from them no caterpillars emerged. In such mines would be found the dried skin of the caterpillar and the larva or pupa of a parasite. Only casual observations were made on the habits and life cycles of the different species of parasites. One of the common species was found to attack the more mature caterpillars and pupate in a small, oval, white cocoon suspended in the tentiform mine. Others destroyed the younger miners and pupated without producing cocoons in the blotch mines. The grub of one of the parasites was observed to attack the miner just behind the third pair of thoracic legs, paralyzing and eventually destroying it.

The collection of parasites was first submitted to Prof. Crosby, who, from a portion of the collection, identified two species: *Sympiesis nigrifemora* Ash. and *S. tischerae* Ash. Later Mr. Girault examined the collection and identified two new species, *S. meteori* Girault and *Eulophus lineaticoxa* Girault, and one previously recognized species, *S. dolichogaster* Ash. Besides these five species, there were a number of males which were not determined. Brunn (1) reared two species of Sympiesis from the mines of this insect. They were recorded under the manuscript names of *S. minutus* Howard and *S. lithocolletidis* Howard; but the descriptions by Howard were apparently never published, and Ashmead later redescribed the latter species as *S. nigrifemora* Ash.

LITERATURE CITED

(1) Brunn, A. E.
1883. Tineidae infesting apple trees at Ithaca. Cornell Univ. Agr. Exp. Sta. 2d Ann. Rpt. 1882/83, p. 148–162, pl. 5–6.

(2) Chambers, V. T.
1873. Micro-Lepidoptera. *In* Canad. Ent., v. 5, no. 3, p. 44–50.

(3) Dietz, W. G.
1907. The North American species of the genus Ornix Tr. *In* Trans. Amer. Ent. Soc., v. 33, no. 2/3, p. 287–297, pl. 4.

(4) Forbes, S. A.
1889. The apple Ornix. *In* 15th Ann. Rpt. State Ent. Ill. [1884/86], p. 51–57.

(5) Jarvis, C. D.
1906. The apple leaf-miner. A new pest of the apple. Conn. Storrs Agr. Exp. Sta. Bul. 45, p. 37–55.

(6) Lowe, V. H.
1900. Two apple leaf miners. *In* N. Y. Agr. Exp. Sta. Bul. 180, p. 131–135, pl. 6–7.

(7) Packard, A. S.
1869. Guide to the study of insects. 702 p., illus., 11 pl. Salem.

PLATE XXXIII

Ornix geminatella Pack.:

Fig. 1.—Moth expanded. × 10.

Fig. 2.—Moth at rest on leaf. × 2½.

Fig. 3.—Egg on lower surface of leaf; also tunnel made by miner on leaving the egg. × 80.

Fig. 4.—Dorsal view of first larval stage; below, side view of head and thorax. × 18.

Fig. 5.—Dorsal view of second larval stage. × 18.

Fig. 6.—Side view of second larval stage. × 18.

Fig. 7.—Dorsal view of third larval stage, showing edge of thoracic legs. × 18.

Fig. 8.—Dorsal view of fourth larval stage. × 18.

Fig. 9.—Side view of fourth larval stage. × 18.

Fig. 10.—Ventral view of pupa. × 18.

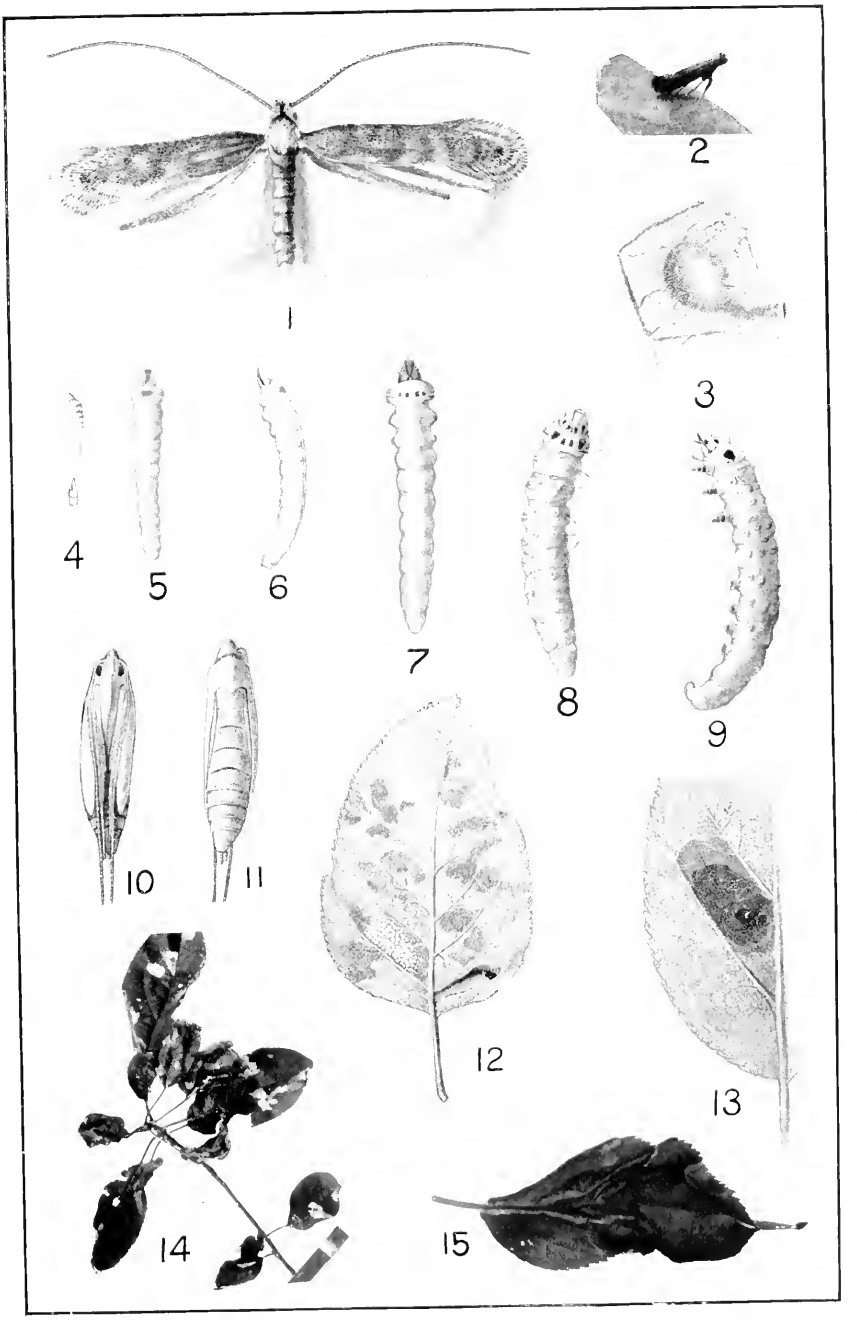
Fig. 11.—Dorsal view of same. × 18.

Fig. 12.—Lower surface of leaf with numerous partly developed mines; also two cocoons, one exposed. The cocoon is usually on the upper surface of the leaf. × 1.

Fig. 13.—Portion of leaf showing a mine in process of development. The serpentine mine was completed on June 24, the small darkly shaded area of the blotch mine June 25, the second area on June 27, the third area on June 29, and on June 30 the blotch was completed and then transformed to the tentiform mine. × 2. Egg more enlarged.

Fig. 14.—A small twig showing leaves badly curled and injured by numerous mines. × ¾.

Fig. 15.—Leaf much distorted with 10 mines almost completed; also one cocoon appears at the tip of the leaf. Natural size.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

10 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

▽

Vol. VI MAY 29, 1916 No. 9

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

EDITORIAL COMMITTEE

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., MAY 29, 1916 NO. 9

A WESTERN FIELDROT OF THE IRISH POTATO TUBER CAUSED BY FUSARIUM RADICICOLA

By O. A. PRATT,

Assistant Pathologist, Office of Cotton and Truck Disease Investigations, Bureau of Plant Industry

INTRODUCTION

Tuber-rots of the Irish potato (*Solanum tuberosum*) which are common to the arid[1] West may be grouped into two classes: Storage-rots and field-rots. This paper is concerned only with certain rots attacking the potato tuber while growing in the field. From the tuber-rots under discussion, the fungus *Fusarium radicicola* Wollenw. was isolated. Carpenter[2] in 1915 demonstrated that *F. radicicola* could, under laboratory conditions, cause decays in potato tubers similar in every way to these rots. His experiments, however, were conducted wholly in the laboratories of the Department of Agriculture, in Washington, D. C. It was therefore thought practicable to present this paper, which gives the results of experiments performed under field conditions in the irrigated West. These experiments substantiate the results obtained by Carpenter and further establish the relationship of *F. radicicola* to the field tuber-rots under consideration.

THE DISEASE

Under the head of fieldrot are considered several types of decay occurring in potato tubers while yet in the field—a stem-end rot, a lenticel rot, and a rot proceeding from eye infections. Eye infections in the field are not as common as stem-end and lenticel infections. These types of rot are known as "stem-end rot," "field dryrot," or "blackrot." The name "blackrot" best describes them, for the decayed tissues are nearly black in color when the tubers are taken from the field. The rot may be further described as a comparatively dry rot, dark to nearly black in color, proceeding from the stem end, lenticels, and occasionally from the eyes of the tuber. The decay is first recognized by the blackened, sunken appearance of the stem end, or, in the case of lenticel and eye

[1] The observations and experiments set forth in this paper were confined principally to southern Idaho.

[2] Carpenter, C. W. Some potato tuber-rots caused by species of Fusarium. *In* Jour. Agr. Research, v. 5, no. 5, p. 183–210, pl. A–B (col.), 14–19. 1915.

infections, by the blackened, more or less sunken spots on the surface of the tuber. Tubers collected in a commercial potato field and infected in this manner are shown in Plate XXXIV, figures 1 to 6. This black color is lost in part as the infection becomes older, the infected tissues taking on various shades from nearly black to sepia brown. In connection with the stem-end rot, the fungus often proceeds down the vascular tissue, killing and blackening the network of bundles. Figures 5 and 6 in Plate XXXIV show sections of a tuber infected in this manner. Often it is possible to break away the cortical tissues and lay bare the blackened network. Lenticel infection proceeds outward in all directions from the point of infection and may or may not extend down to the main vascular system. Very frequently in the case of eye infections the vascular strand connecting the eye with the main vascular system is blackened, but it is seldom that such infection extends far into the main vascular ring. Blackrot is confined principally to potatoes of the Idaho Rural, Pearl, and other round types.

Closely related to the blackrot of potatoes of the round types is a jelly-end rot attacking principally varieties of the Burbank group. Jelly-end-infected tubers of the Netted Gem variety are shown in Plate XXXV, figures 1 to 3. The jelly-end rot of the Burbank group differs from the blackrot of round types of potatoes in that it is a softrot, light to dark brown in color, while the blackrot is a comparatively dry rot, black or nearly black in color. Jelly-end rot may be described as a soft, wet rot of the tubers proceeding from the stem end downward through the tuber attacking all tissues but apparently advancing somewhat more rapidly through the vascular bundles. Examination of tubers infected with jelly-end rot, however, often reveals no perceptible discoloration of the vascular tissue below the line of the rot in the other tissues. As the decay becomes older, the stem end becomes somewhat shriveled and dried, often closely resembling the type of decay caused in storage by *F. trichothecioides* Wollenw.[1] Lenticel and eye infections are seldom found in connection with the jelly-end rot of the Burbank group.

Occasionally a softrot of the seed end is also found. A Netted Gem tuber infected at both the seed end and the stem end is shown in Plate XXXV, figure 1. *F. radicicola* was isolated from both ends of this tuber. There was apparently no infection in the vascular tissues connecting the two regions of decay.

At first it was thought that the jelly-end rot of the Burbank group and the blackrot of round types of potatoes were two distinct diseases, but inoculations made in 1914 into the stem ends of Netted Gem and Idaho Rural tubers with *F. radicicola* led to the belief that they might be caused by the same organism. Material collected in the field, whether jelly-end rot or blackrot, when placed in a moist chamber for a few days

[1] Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by Fusarium trichothecioides, Wollenw. *In* Jour. Washington Acad. Sci., v. 2, no. 6, p. 146–152, illus. 1912.

usually showed tufts of *F. radicicola*. Infected tubers of Idaho Rural potatoes kept 10 days in a moist chamber at room temperature are shown in Plate XXXV, figures 4 and 5. Tufts of *F. radicicola* have appeared. Inoculations in 1915 left no doubt in the writer's mind that *F. radicicola* was capable of causing both types of rot.

DISTRIBUTION AND ECONOMIC IMPORTANCE

F. radicicola is apparently widely distributed. Wollenweber [1] states that its habitat is "on partly decayed tubers and roots of plants, such as *Solanum tubcrosum* in Europe and America (collected by Wollenweber) and *Ipomoea batatas* in the United States of America (collected by Harter and Field)." Carpenter [2] makes the following statement as to its habitat: "On partly decayed tubers and roots of plants. Cause of potato dryrot and jelly-end rot. Identified from the following: *Ipomoea batatas* (collected by Mr. L. L. Harter); *Musa sapientum* (collected by Mr. S. F. Ashby, Jamaica, Porto Rico); *Cucumis sativus* (collected by Mr. F. V. Rand, West Haven, Conn.); soil (collected by Mr. F. C. Werkenthin, Austin, Tex.)."

The writer has isolated *F. radicicola* from the roots of poplar trees (*Populus deltoides*) at Jerome, Idaho, where he found it associated with crownrot. The fact that the fungus appears on potato tubers when disease-free seed potatoes are planted on raw desert lands suggests that it may be well distributed throughout the desert soils. Orton [3] in 1909 reported jelly-end rot of potatoes from the San Joaquin Valley, in California.

F. radicicola has been reported on potatoes from Idaho, Oregon, and California by Wollenweber [4] and from Idaho, Oregon, California, Nevada, Mississippi, New York, Virginia, and the District of Columbia by Carpenter.[5] The writer has isolated this fungus from decayed potato tubers from the following localities in Idaho: Idaho Falls, Blackfoot, Aberdeen, Rupert, Murtaugh, Twin Falls, Filer, Kimberly, Jerome, Wendell, Gooding, King Hill, and Caldwell, and has observed the rot in potato fields in many other localities in the State. The disease apparently appears at its worst under dry-land-farming conditions and in raw desert land planted to potatoes before having been in other crops. On comparing rotted tubers collected by himself in Idaho with specimens sent to the Department of Agriculture from California and Oregon he was convinced that the rots were of one and the same nature. He has also observed rots identical in outward appearance with those found in Idaho, in Portland, Oreg., Seattle, Wash., and British Columbia.

[1] Wollenweber, H. W. Identification of species of Fusarium occurring on the sweet potato, Ipomoea batatas. *In* Jour. Agr. Research, v. 2, no. 4, p. 257. 1914.

[2] Carpenter, C. W. Op cit., p. 206.

[3] Orton, W. A. Potato diseases in San Joaquin County, Cal. U. S. Dept. Agr., Bur. Plant Indus. Circ. 23, 14 p. 1909.

[4] Wollenweber, H. W. Op. cit.

[5] Carpenter, C. W. Op. cit.

In the irrigated portions of Idaho the economic importance of the disease has varied greatly from year to year. In 1913 the writer was usually able to find only an occasional rotted tuber in any one commercial field. In a few fields which had been planted on raw desert land and poorly cared for he found as high as 80 per cent of the tubers infected with stem-end blackrot and lenticel rot. The year 1914 might be called an epidemic year. In one 50-acre field of Netted Gems near Jerome, Idaho, he found as high as 40 per cent of the crop infected with jelly-end rot. Similar conditions were observed in many other fields in the irrigated portions of southern Idaho. Stem-end blackrot and lenticel rot were also found very abundant in the fields of Idaho Rurals. It is significant that in 1914 a freeze occurred in June which killed the vines to the ground, the plants coming up anew and producing a crop. Often the origin of infection could be traced from the frozen tip of the vine down through the stem to the infected tubers. Although infected tubers were found in most of the commercial fields visited in 1915, the disease this year was of slight importance.

EXPERIMENTAL WORK

PRELIMINARY EXPERIMENT IN 1914

In the fall of 1914 ten Idaho Rural tubers and ten Netted Gem tubers were disinfected by dipping in formaldehyde and were punctured at the stem end with a needle carrying spores from a culture of *F. radicicola* which had been isolated from a potato tuber infected with blackrot. After inoculation the tubers were placed in moist chambers, where they remained for something over a month. An examination of the tubers showed that infection had been produced in every tuber inoculated. The infection in the Idaho Rurals was similar in all respects to the blackrot occurring in the field. The infection in the Netted Gems was not quite so dark in color as that produced in the Idaho Rurals and resembled certain stages of jelly-end rot collected in the field. No checks were prepared.

LABORATORY EXPERIMENTS IN 1915

On August 6, young and apparently healthy potato tubers of the Netted Gem and Idaho Rural varieties were selected, carefully washed, and disinfected in a solution of formaldehyde (1:240). After disinfection the tubers were dried and inoculated with *F. radicicola*. The methods of inoculation were as follows: (1) By spraying with a spore suspension; (2) by wounding the tubers with a needle bearing spores; and (3) by dipping the broken stolon ends in a spore suspension. In method 3 the tubers were taken from the field with their stolons attached. After disinfection each stolon was broken off afresh at from 1 to 2 inches from its junction with the tuber and inoculated as stated in the foregoing.

Fifty tubers each of Idaho Rural and Netted Gem, respectively, were inoculated by methods 1 and 2, and twenty-five tubers each of Idaho Rural and Netted Gem were inoculated by method 3. Checks on each experiment were prepared in the same manner, except that in method 1 the tubers were sprayed with sterile water, in method 2 the tubers were wounded with a sterile needle, and in method 3 the broken stolon ends were dipped in sterile water. Inoculated tubers and checks were placed in moist chambers and put in the culture room of the Experiment Station laboratory. During the course of these experiments the culture-room temperature varied from a minimum of 20° to a maximum of 29° C. Temperatures were taken daily at 8.30 a. m. and 5.30 p. m. After a month the tubers were examined. Table I gives a summary of the experiments and the number of tubers found infected.

TABLE I.—*Summary and results of laboratory inoculations of Solanum tuberosum*

Method No.	Method of inoculation and parts inoculated.	Variety.	Number of tubers inoculated.	Number of tubers infected.
1	Tubers sprayed with suspension of spores	Netted Gem	50	48
		Idaho Rural	50	50
	Check. Tubers sprayed with sterile water	Netted Gem	50	0
		Idaho Rural	50	0
2	Tubers punctured with inoculated needle at stem end	Netted Gem	50	50
		Idaho Rural	50	50
	Check. Tubers; stem end punctured with sterile needle.	Netted Gem	50	0
		Idaho Rural	50	0
3	Tubers; broken stolon ends dipped in spore suspension.	Netted Gem	25	25
		Idaho Rural	25	19
	Check. Tubers; broken stolon ends dipped in sterile water.	Netted Gem	25	0
		Idaho Rural	25	0

Of the 50 Netted Gem tubers sprayed with the spore suspension, 48 showed infection. Stem-end infection was present in each of the inoculated tubers. Lenticel infections were present on most of the tubers, and eye infections were also found. Every Idaho Rural tuber sprayed with the spore suspension showed infection at the stem end. The majority showed lenticel infections and several showed eye infections. Lenticel infections, induced by spraying with the spore suspension, are shown in Plate XXXVI, figure 3. In figure 4 of Plate XXXVI is shown the same tuber after remaining several days longer in the moist chamber. Tufts of *F. radicicola* have appeared over the surface of the decayed areas.

A stem-end infection of an Idaho Rural tuber sprayed with the spore suspension is shown in Plate XXXVI, figure 5. Every tuber, whether Netted Gem or Idaho Rural, developed infection when punctured at the stem end with a needle carrying the spores of the fungus. Decays induced in this manner are shown on Plate XXXVI, figures 1 and 2. Twenty-five stem-end tuber infections resulted from the inoculation of the broken stolon ends in the Netted Gems, and 19 in the Idaho Rurals. The decay resulting from this method of inoculation was similar in every

way to that produced by the other methods. A stem-end infection resulting from the inoculation of the broken stolon end under laboratory conditions is shown in Plate XXXVI, figure 6. In Plate XXXVI, figure 7, is shown an Idaho Rural tuber cut to expose the blackening of the vascular tissue which resulted from the inoculation of the tuber stolon. None of the checks were infected. The fungus was recovered from the decayed tissues each time the attempt was made.

EXPERIMENTS IN THE FIELD IN 1915

On August 11, in a plot in which disease-free Idaho Rural and Netted Gem seed potatoes had been planted, apparently healthy potato plants were selected. The soil was removed from around the plants in such a manner as to expose the tubers without disturbing their position. Three growing tubers under each plant were then inoculated with *F. radicicola*, after which the soil was replaced, care being exercised to place moist soil next to the tubers. The methods of inoculation were, respectively, as follows: (1) By spraying the tubers with a spore suspension; (2) by wounding each tuber stolon with a needle bearing spores at from 1 to 2 inches from its junction with the tuber; (3) by wounding the upper surface of each tuber with a needle bearing spores, and (4) by puncturing each tuber at the stem end with a spore-bearing needle. Ten plants each of Idaho Rural and Netted Gem potatoes were used in each experiment. As a check on each experiment, a similar number of apparently healthy Idaho Rural and Netted Gem plants were selected and a similar number of growing tubers treated in the same manner, except that in the case of experiment 1 the tubers were sprayed with sterile water, and in numbers 2, 3, and 4 a sterile needle was used in place of a spore-bearing needle.

A fifth experiment was set up in which 10 apparently healthy Idaho Rural and 10 apparently healthy Netted Gem plants, growing in the same plot with those employed in the four experiments just described, were used. In this experiment, the stem of each plant was punctured at the crown with a needle carrying spores of *F. radicicola*. Checks were prepared in the same manner, except that the stem of each plant was punctured with a sterile needle.

The soil of the plot in which these experiments were made was very dry and no irrigation water could be applied after the inoculations were made. During the course of the experiments (August 11 to September 6) the minimum soil temperature recorded was 66° and the maximum 84° F. The soil temperature was taken at a depth at which the potato tubers were found lying by burying the bulb of a soil thermograph under a potato plant. A little less than a month after making the inoculations an examination of all the plants was made. Table II gives a summary of the experiments and the results obtained from inoculating growing potato plants and tubers with *F. radicicola*.

TABLE II.—*Summary and results of inoculating growing potato plants and tubers with Fusarium radicicola*

Experiment No.	Method of inoculation.	Variety.	Number of inoculations.	Number of tubers infected.
1	Tubers sprayed with suspension of spores	Idaho Rural	30	15
		Netted Gem	30	17
	Check. Tubers sprayed with sterile water	Idaho Rural	30	0
		Netted Gem	30	0
2	Tuber stolons punctured with inoculated needle	Idaho Rural	30	27
		Netted Gem	30	23
	Check. Tuber stolons punctured with sterile needle	Idaho Rural	30	0
		Netted Gem	30	0
3	Tubers punctured with inoculated needle	Idaho Rural	30	30
		Netted Gem	30	30
	Check. Tubers punctured with sterile needle	Idaho Rural	30	0
		Netted Gem	30	0
4	Tubers punctured at stem end with inoculated needle	Idaho Rural	30	30
		Netted Gem	30	30
	Check. Tubers punctured at stem end with sterile needle	Idaho Rural	30	0
		Netted Gem	30	0
5	Stem of plant punctured at crown with inoculated needle	Idaho Rural	10	0
		Netted Gem	10	0
	Check. Plant stem punctured at crown with sterile needle	Idaho Rural	10	0
		Netted Gem	10	0

Of the 30 Idaho Rural tubers sprayed, 15 showed infection with stem-end and lenticel rot. Of the 30 Netted Gem tubers sprayed, 17 showed stem-end rot. Lenticel rot did not occur on all of the Netted Gem tubers and where it did occur the infections were very slight. The thicker skin of the Netted Gem probably renders it more resistant to fungus attacks than the Idaho Rural. The failure of a part of the sprayed tubers to develop infection can probably be attributed to the extremely dry condition of the soil. Infections resulting from spraying the growing tubers with a suspension of the spores of *F. radicicola* are shown in Plate XXXVI, figures 1 to 4. In figure 4, Plate XXXVI, is shown an eye infection which has extended down into the vascular system. *F. radicicola* was recovered from the discolored vascular tissue of this tuber. None of the checks showed any infection. Twenty-seven Idaho Rural tubers infected with stem-end rot resulted from the puncturing of the 30 tuber stolons. The three which failed to develop infection were under the same plant. Twenty-three of the Netted Gem tubers whose stolons were inoculated showed stem-end infection. Seven showed no evidence of infection in the tubers, though the stolons were black and dead up to within about one-eighth of an inch of their juncture with the tubers. Where infection in the tuber was found the line of infection could easily be traced down the stolon from the point of inoculation into the tuber.

Tuber infections resulting from the inoculation of the stolons in the field are shown in Plate XXXVII, figures 5 to 8. Both stem-end rot and vascular infection are shown. Figure 8, Plate XXXVII, represents a Netted Gem tuber with stem-end infection resulting from the inoculation of the

stolon. The rot in this case was nearly black in color, soft, and resembled the earlier stages of the jelly-end rot often found in commercial fields. Vascular infection also developed in this tuber. The fungus was recovered from all infected tissues whenever the attempt was made. None of the checks were infected. Infection resulted in all cases where tubers were punctured with a needle carrying the spores of the fungus. None of the checks were infected. In the case of the checks the punctures could be seen easily but were healed over in each case. The inoculations made into the stems of potato plants failed to give very decisive results. In each case a blackening of the tissue adjacent to the puncture was observed. This blackening extended up and down from the point of puncture for from one-eighth to one-half an inch and in most cases also extended into the pith.

BLACKROT

The infections, whether at the stem end, at the lenticels, or at the eyes, produced by the artificial inoculation of Idaho Rural tubers with *F. radicicola*, could not be distinguished in any way from the infections on decayed tubers collected in the commercial fields. The infections resulting from the inoculation of growing tubers in the station plots when final examination was made were not as deep or as far advanced as many infections occurring naturally in the field, but this can easily be explained by the late date at which the inoculations were made. In fact, at the time the inoculations were made, tubers with well-advanced decay were being found in commercial fields. On the other hand, tubers with decay no farther advanced than that resulting from the inoculations have often been found in the field late in the season. In every case where an attempt was made, the fungus was recovered.

Tubers infected by inoculation in the field, by spraying with the spore suspension, by the puncture of the tuber with an inoculating needle, and by puncture of the tuber stolons, were placed in moist chambers, and in each case, after a few days, tufts of *F. radicicola* appeared. Blackrot-infected tubers in commercial fields, after being kept in a moist chamber from 3 to 10 days at temperatures ranging from 65° to 75° F., invariably threw out tufts of this fungus (Pl. XXXV, fig. 4, 5). Isolations made from the cortical and medullary tissues of blackrot-infected tubers have never yielded any fungus other than *F. radicicola*, which could be considered as the cause of the disease. Isolations made from stem-end blackrot-infected Idaho Rurals, Pearls, and other round types of potatoes have occasionally yielded *F. oxysporum*, especially when the culture was made from or near the vascular tissue. The failure to obtain *F. oxysporum* from lenticel and eye infections of tubers collected in commercial fields leads the writer to conclude that when *F. oxysporum* is found in stem-end infections it probably entered as a vascular parasite, independ-

ent of *F. radicicola*. *F. oxysporum* has never been found in connection with the stem-end blackrot of western potatoes to the exclusion of *F. radicicola*.

Fully 50 per cent of all cultures made from the decayed cortical and medullary tissues of tubers infected with stem-end and lenticel rot have remained sterile. This may have been due to improper cultural conditions, but it is believed that the discoloration of the tuber tissue often extends some distance beyond the point actually reached by the invading fungus. Stem-end blackrot-infected tubers often show a black net necrosis. Isolations made from the black network of bundles, if made some distance below the stem end, often fail to reveal any fungus. On the other hand, many such cultures have revealed *F. radicicola*, and occasionally both *F. radicicola* and *F. oxysporum*. That *F. radicicola* is capable of causing the blackened net, as well as the stem-end blackrot, is fully demonstrated by the results of artificial inoculations Pl. (XXXVI, fig. 7, and Pl. XXXVII, fig. 6, 8), though the fungus may not always be present throughout the entire length of the blackened bundle area.

JELLY-END ROT

Whenever the inoculation of Netted Gem tubers took effect at the stem end, an infection typical of certain types of jelly-end rot found in the commercial fields was produced. In the moist chamber under laboratory conditions infections at the stem end induced by puncturing the tubers, by spraying with a spore suspension, or by puncture of the stolons with an inoculating needle were fairly typical of the advanced stages of jelly-end rot, being soft and watery. Under field conditions, infections at the stem end induced by spraying the tubers with the spore suspension, by puncturing with an inoculating needle, or by the inoculation of the stolons were in no case as pronounced as the infections found occurring naturally in the field. Those induced by a puncture at the stem end were deeper than those produced by the other methods.

The failure of the inoculations in the field to develop as severe cases of infection as those occurring in nature may be attributed to the late date on which the inoculations were made and to the very dry condition of the soil. Aside from the depth of the infection at the stem end, the stem-end decays induced by artificial inoculation were very similar in appearance to infections found occurring naturally in commercial fields of Netted Gem potatoes. Wherever the attempt was made, *F. radicicola* was recovered from the stem-end infections induced by the inoculations. It is evident, therefore, that *F. radicicola* is capable of producing a jelly-end rot of the potato tuber. However, isolations made from such rotted tubers taken from the field have not always revealed *F. radicicola* to the exclusion of other fungi. *F. oxysporum* is frequently obtained.

Wollenweber[1] reports the isolation of *F. orthoceras* from jelly-end tubers and thought it the probable cause of the disease. The writer has twice isolated *F. trichothecioides* from such tubers fresh from the field.

Artificial infection of the growing tuber with *F. trichothecioides* under western conditions has never been accomplished. Under conditions of high humidity Jamieson and Wollenweber[2] were able to produce an infection in the growing tuber with this fungus, but their results are not believed to be indicative of what actually takes place in nature in the irrigated West. Tubers infected with jelly-end rot, when kept in a moist chamber for a few days, invariably threw out tufts of *F. radicicola* through the lenticels, although from these same tubers with well-advanced stem-end rot other fungi, notably *F. oxysporum*, have been isolated from the interior of the tuber. Carpenter[3] has shown that *F. oxysporum* is capable of producing a similar rot of the potato tuber, and from its frequent occurrence in connection with jelly-end-rot-infected tubers it must be considered as one of the factors involved in producing this type of rot. Other Fusarium species, either independently or in conjunction with *F. radicicola*, may be in part responsible for the disease.

STORAGE EXPERIMENTS

In the fall of 1914 two ordinary 2-bushel sacks filled with Netted Gems infected with jelly-end rot were secured. With a soft blue pencil, a line was drawn around each tuber in such a manner that the blue line separated the decayed from the healthy tissue. The tubers were then sacked and put in storage in the potato cellar of the Jerome Experiment Station, at Jerome, Idaho. Fifty tubers each of Pearls and Idaho Rurals infected with stem-end and lenticel blackrot were secured. On each tuber a blue line was drawn around the stem end at the margin of the infected and healthy tissues. Lenticel infections were marked in the same manner. The marked Pearl and Idaho Rural tubers were then sacked and placed in storage near the similarly treated Netted Gems infected with jelly-end rot.

The storage period was from November 15, 1914, to April 12, 1915. The temperature of the cellar during this period ranged from 32° to 48° F. During the last six weeks of the storage period the minimum temperature was 36°, and for the greater part of this time the temperature approached the maximum of 48°. On April 12 the tubers were removed from the sacks and examined one by one to determine whether the rot had continued to develop. In no case could any perceptible advance in the decay be found. It is apparent that neither jelly-

[1] Wollenweber, H. W. Studies on the Fusarium problem. *In* Phytopathology, v. 3, no. 1, p. 24–50. 1 fig., pl. 5. 1913.

[2] Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by Fusarium trichothecioides, Wollenw. *In* Jour. Washington Acad. Sci., v. 2, no. 6, p. 146–152, illus. 1912.

[3] Carpenter, C. W. Op. cit.

end rot nor blackrot makes any progress in storage at a temperature of 48° or under.

This conclusion is further substantiated by results obtained in storing several sacks of blackrot-infected Idaho Rural and Pearl tubers for experimental use in the fall of 1913. Although the infected stock remained in the cellar until the middle of May, 1914, when the cellar temperatures had risen to something over 50° F., the tubers were apparently as sound as at the time they were put in storage. Carpenter [1] has found that when tubers were inoculated with *F. radicicola* and kept at a temperature of 12° C. (approximately 53° F.) no rot developed.

EFFECT OF PLANTING INFECTED SEED

In the spring of 1915 three plots were planted with infected seed potatoes. Plot 1 was planted with Idaho Rural potatoes every seed piece of which showed infection with *F. radicicola*, stem-end blackrot, or lenticel rot. The presence of the fungus was verified by artificial cultures. Plot 2 was planted with Pearl potatoes every seed piece of which was infected with *F. radicicola*, stem-end blackrot, or lenticel rot, the presence of the fungus being verified by artificial cultures. Plot 3 was planted with Netted Gem potatoes infected with jelly-end rot. The seed pieces were cut from the stem end, care being exercised to see that at least one healthy eye was present on each seed piece. Cultures from this seed gave a variety of fungi, including *F. radicicola* and *F. oxysporum*. Check plots were planted with the same varieties. The seed selected for the check plots was entirely free from disease and was disinfected for 1½ hours in a solution of mercuric chlorid (4 ounces of mercuric chlorid to 30 gallons of water). All of the plots were planted on alfalfa land which had never before been planted to potatoes. The soil was a heavy clay loam of lava-ash formation. Irrigation was given on July 4 and 5, July 16, July 31, and August 1. Throughout the season the plots were kept in a good state of tilth, but they suffered somewhat from lack of moisture during the latter part of August. Table III shows the percentage of disease in the harvested product.

TABLE III.—*Percentage of disease in harvested potatoes*

Plot No.	Variety.	Condition of seed.	Percentage of disease in tubers.	
			Vascular infection.	Tuber-rots.
1	Idaho Rural	Infected with blackrot	96	82
2	Pearl	do	44	40
3	Netted Gem	Infected with jelly-end rot	16	0
4	Idaho Rural	Disease-free, disinfected	40	0
5	Pearl	do	14	1
6	Netted Gem	do	10	0

[1] Carpenter, C. W. Op. cit.

The vascular infection present in plots 1 and 2 was all of the heavy black type demonstrated to be caused by *F. radicicola.* Numerous cultures from the vascular systems of tubers from these plots gave the fungus. The percentages of rot include all phases of blackrot, including stem-end, lenticel, and eye infections. Strangely enough, no tuber-rots developed in plot 3. Of the tubers from plot 3, 16 per cent showed vascular infection, of which 14 per cent were of the type usually ascribed to *F. oxysporum* and 2 per cent were of the black type caused by *F. radicicola.* Cultures made from the vascular systems of infected tubers in this plot give *F. oxysporum* in all cases of light-brown discoloration and *F. radicicola* in all cases of black vascular discoloration. In the check plots, 1 per cent of blackrot appeared in plot 5. The others were free from all tuber-rots. The vascular infection present in the check plots was for the most part of the type ascribed to *F. oxysporum.* A few tubers showing blackened vascular bundles were found, and *F. radicicola* was isolated from such tissues whenever the attempt was made.

The results clearly show that seed infected with blackrot will produce infection in the resulting product. From the fact that no jelly-end rot resulted from planting jelly-end-infected seed, the conclusion should not be drawn that such seed can not cause infection in the resulting product, but rather that it requires conditions for its development different from those required for the development of blackrot.

CONTROL OF BLACKROT

Absolute control of blackrot will be difficult. When potatoes are planted on alfalfa or grain lands blackrot is rarely found if the crop has had sufficient water to make good growth conditions possible. Plantings of disease-free seed potatoes on raw desert lands in 1915 gave as high as 11 per cent of tubers infected with blackrot in the harvested product, whereas plantings of disease-free tubers on alfalfa or grain lands were usually free from the disease, although as high as 5 per cent of infected potatoes were found in the harvested product of one plot on alfalfa land. Judging from the results of three years' observations in commercial fields, it is apparent that losses from blackrot can be reduced to a minimum by planting only on land which has been in cultivation for a number of years and by giving the growing crop the proper amount of water, care, and attention. The crop should be kept in a good growing condition until maturity or frost. Jelly-end rot, on the other hand, has been found in fields where all the conditions of growth were apparently ideal. Some adverse condition, however, is probably responsible for its development. Further research upon jelly-end rot and its cause and occurrence is highly desirable.

Both jelly-end rot and blackrot-infected tubers may be stored with safety, provided the storage cellar is fairly well ventilated and the temperature kept below 50° F.

SUMMARY

(1) *Fusarium radicicola* Wollenw. is the cause of a field blackrot of potato tubers in southern Idaho. The disease is confined principally to potatoes of the round type, such as Idaho Rural and Pearl.

(2) *F. radicicola* is capable of causing a jelly-end rot of potatoes similar to the jelly-end rot of the Burbank group found in southern Idaho, but under actual field conditions other factors are apparently in part responsible.

(3) Neither blackrot nor jelly-end rot makes any progress in storage at or below a temperature of 50° F.

(4) Seed pieces infected with blackrot will bring about infection in the following crop.

(5) *F. radicicola* is apparently well distributed throughout the desert soils.

(6) Blackrot may be controlled fairly well by planting potatoes only on lands which have been in other crops for a number of years and by providing good conditions for growth.

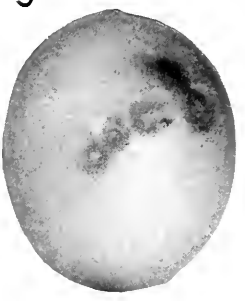
PLATE XXXIV

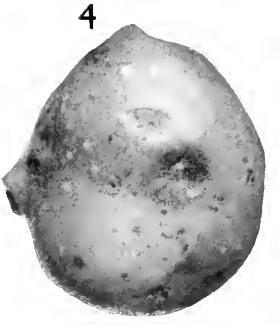
Fig. 1, 2, 3, 4.—Types of stem-end blackrot, lenticel rot, and eye rot in Idaho Rural potato tubers. Field material.

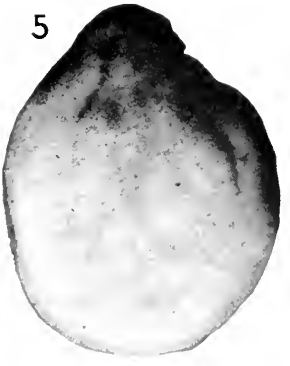
Fig. 5, 6.—Longitudinal and cross sections of an Idaho Rural tuber infected with blackrot. Note the blackened vascular system. Field material.

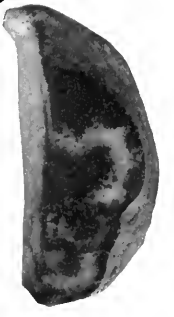

1


2

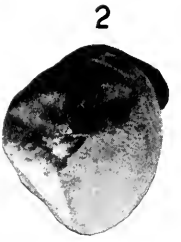

3


4

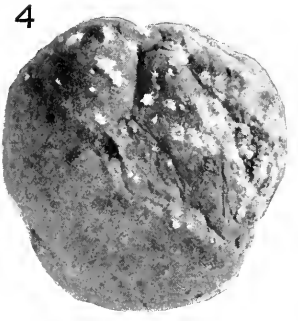

5


6


1


2


3


4


5

PLATE XXXV

Fig. 1.—Netted Gem potato tuber infected with jelly-end rot. A soft bud-end infection may also be seen. Field material.

Fig. 2.—Stem-end view of a Netted Gem tuber infected with jelly-end rot. Field material.

Fig. 3.—Longitudinal section of a Netted Gem tuber infected with jelly-end rot. Field material.

Fig. 4.—Idaho Rural tuber infected with stem-end and lenticel blackrot, after having been kept 10 days in a moist chamber. Tufts of *Fusarium radicicola* have appeared. Field material.

Fig. 5.—Idaho Rural tuber infected with lenticel blackrot after having been kept in a moist chamber for 10 days. A single tuft of *F. radicicola* has appeared. Field material.

PLATE XXXVI

Fig. 1, 2.—Stem-end blackrot produced by stem-end punctures with a needle carrying *Fusarium radicicola*. Netted Gem and Idaho Rural potato tubers. Laboratory inoculations.

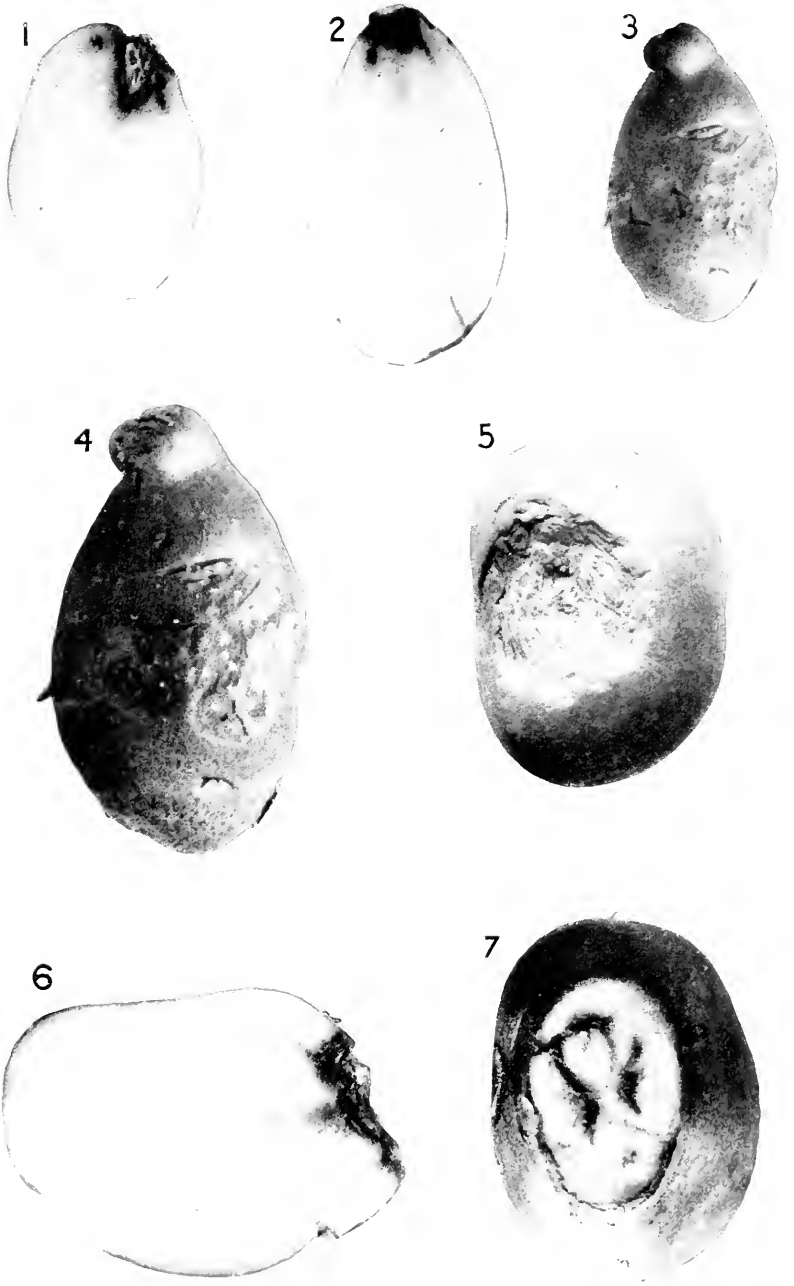
Fig. 3.—Lenticel blackrot produced by spraying the tuber with a spore suspension of *F. radicicola*. Netted Gem tuber. Laboratory inoculation.

Fig. 4.—Same tuber as shown in figure 3; after having been kept a few days longer in the moist chamber. Note the tufts of *F. radicicola* that have appeared.

Fig. 5.—Stem-end blackrot produced by spraying an Idaho Rural tuber with a spore suspension of *F. radicicola*. Laboratory inoculation.

Fig. 6.—Stem-end blackrot produced by the inoculation of the tuber stolon. Idaho Rural tuber. Laboratory inoculation.

Fig. 7.—Blackened vascular system produced by the inoculation of the tuber stolon. Idaho Rural tuber. Laboratory inoculation.


1
2
3
4
5
6
7

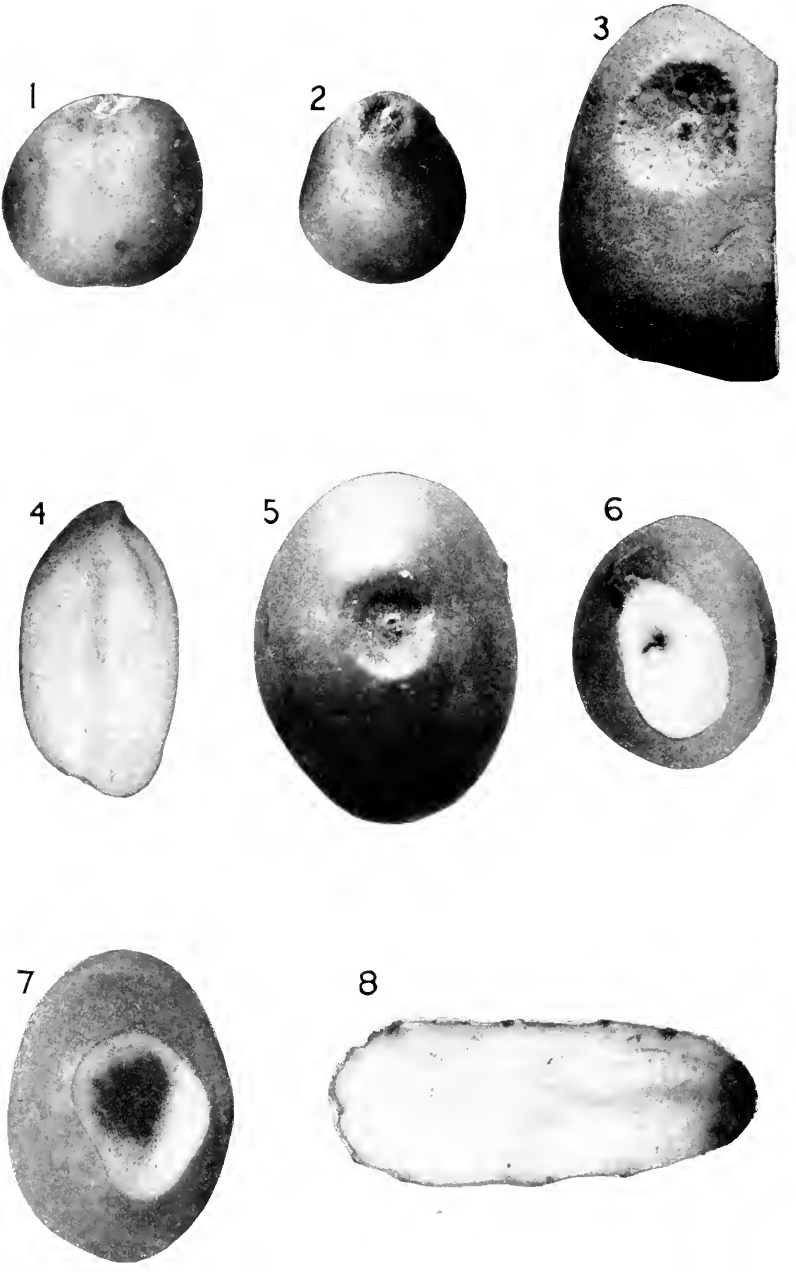

1
2
3
4
5
6
7
8

PLATE XXXVII

Fig. 1, 2, 3.—Stem-end and lenticel blackrot produced by spraying the growing tubers with a spore suspension of *Fusarium radicicola*. Idaho Rural potato tubers. Field inoculations.

Fig. 4.—Eye infection produced by spraying the growing tuber with a spore suspension of *F. radicicola*. Netted Gem tuber. Field inoculation.

Fig. 5, 6, 7.—Stem-end blackrot produced by the inoculation of the stolons of growing Idaho Rural tuber. Field inoculation.

Fig. 8.—Stem-end rot of Netted Gem tuber produced by inoculating the stolon of the growing tuber.

36290°—16——2

COMPARATIVE STUDY OF THE ROOT SYSTEMS AND LEAF AREAS OF CORN AND THE SORGHUMS

By EDWIN C. MILLER,[1]

Assistant Plant Physiologist, Department of Botany, Kansas Agricultural Experiment Station

INTRODUCTION

During the summers of 1914 and 1915 a series of investigations was conducted to determine the fundamental characteristics possessed by the sorghum plants (*Andropogon sorghum*) which enable them to withstand severe climatic conditions better than the corn plant (*Zea mays*). The results of these investigations will be reported in a series of articles as rapidly as the data are assembled. This paper deals with the comparative study of the root systems and leaf areas of corn, Blackhull kafir, and Dwarf milo. These experiments were carried on at the State Branch Experiment Station at Garden City, Kans. This Station is located in the southwestern part of the State, in latitude 37° 58′ north and longitude 100° 55′ west (Greenwich), and has an elevation of 2,940 feet.

EXPERIMENTAL METHODS

CLIMATIC DATA

The instruments for obtaining the weather data consisted of a thermograph, a hydrograph, a soil thermograph, maximum and minimum thermometers, a psychrometer, a rain gauge, an evaporation tank, and two anemometers. The maximum and minimum thermometers, thermograph, and hydrograph were kept in a standard shelter 4 feet above the ground. One of the anemometers measured the wind velocity at a height of 2 feet and the other at a height of 8 feet. The 2-foot anemometer was connected with a clock attachment so that the wind velocity for each hour was recorded. The bulb of the soil thermograph was buried to a depth of 1 foot.

A portion of the weather records for the growing seasons of 1914 and 1915, grouped in 5-day periods, is given in Table I. This table shows that the climatic conditions of 1914 and 1915 were in marked contrast. The total rainfall for the year 1914 amounted to only 9.7 inches, while that for 1915 totaled 26.77 inches.

[1] Acknowledgments are due Messrs. J. G. Lill and C. B. Brown, of the United States Department of Agriculture, for their aid in obtaining the weather and soil data, and to Mr. M. C. Sewell, formerly superintendent of the Experiment Station at Garden City, Kans., for general assistance in this work.

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for the growing months of 1914 and 1915*

Year and month.	Days (inclusive).	Air temperature (° F.).					Precipitation.	Evaporation.	Wind velocity per hour.
		Average of—			Maximum.	Minimum.			
		Mean.	Maximum.	Minimum.					
1914.							*Inches.*	*Inches.*	*Miles.*
May	1–5	58	68	47	78	44	1.40	0.953	9.0
Do	6–10	65	78	51	92	41	.19	1.484	11.8
Do	11–15	53	61	44	72	38	.20	1.135	10.9
Do	16–20	62	68	55	79	50	.72	.596	13.6
Do	21–25	72	84	59	90	57	.12	1.584	10.2
Do	25–31	69	79	57	89	49	1.00	1.294	6.9
June	1–5	76	87	65	92	62	.19	1.432	13.0
Do	6–10	77	89	64	91	51	.21	1.728	15.2
Do	11–15	76	88	63	96	59	.61	1.520	9.3
Do	16–20	76	89	62	99	58	.39	1.409	6.4
Do	21–25	82	94	69	98	64	.....	1.991	9.9
Do	26–30	77	94	59	103	51	.04	1.862	7.7
July	1–5	74	85	62	94	53	.15	1.200	6.1
Do	6–10	77	91	60	93	53	.10	1.440	4.7
Do	11–15	86	99	69	103	64	.....	1.822	5.7
Do	16–20	76	87	62	101	58	.21	1.416	7.7
Do	21–25	81	94	65	98	64	.10	1.451	5.7
Do	26–31	83	98	66	102	64	T.	2.074	5.7
August	1–5	77	93	65	95	61	.38	1.477	6.1
Do	6–10	77	91	62	95	56	T.	1.792	8.0
Do	11–15	77	91	62	95	58	.19	1.474	7.0
Do	16–20	82	99	64	102	62	.06	1.959	8.2
Do	21–25	77	91	61	99	50	.01	1.745	7.5
Do	25–31	73	87	60	94	54	T.	1.563	7.4
September	1–5	77	94	60	103	55	.....	1.739	7.5
Do	6–10	79	96	64	102	59	.01	1.501	8.6
Do	11–15	75	89	58	96	48	.03	1.653	11.4
Do	16–20	77	90	60	97	56	.....	1.390	7.6
Do	21–25	63	80	44	85	37	.11	1.343	6.4
Do	26–30	67	86	51	90	47	.....	1.740	11.1
1915.									
May	1–5	53	65	38	76	31	.79	1.187	10.0
Do	6–10	56	69	44	81	32	.....	.985	7.7
Do	11–15	71	87	55	94	46	.....	1.857	10.8
Do	16–20	46	55	39	68	32	2.38	1.324	12.2
Do	20–25	67	78	57	90	44	.07	1.069	8.6
Do	25–31	55	65	47	72	39	1.15	1.169	8.1
June	1–5	65	75	58	81	55	.64	.738	8.7
Do	6–10	64	78	52	86	36	.94	1.386	8.6
Do	11–15	66	78	53	87	50	.....	1.490	8.0
Do	16–20	71	85	61	95	56	.07	1.485	8.8
Do	21–25	69	79	58	91	56	.62	1.181	8.5
Do	26–30	72	84	59	88	57	.69	1.419	7.1
July	1–5	66	77	55	83	49	.57	1.451	8.8
Do	6–10	76	90	60	96	54	.51	1.732	8.6
Do	11–15	81	97	67	101	64	.06	1.743	6.7
Do	16–20	72	84	62	96	56	.15	1.407	7.0
Do	21–25	74	85	61	91	56	.13	1.397	5.5
Do	25–31	75	74	64	90	62	.24	1.528	6.8
August	1–5	69	83	56	90	51	.90	1.012	5.8
Do	6–10	70	80	60	94	56	5.11	.860	4.9
Do	11–15	72	83	61	86	59	.10	.927	2.7

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for the growing months of 1914 and 1915*—Continued

Year and month.	Days (inclusive).	Air temperature (° F.).					Precipitation.	Evaporation.	Wind velocity per hour.
		Average of—			Maximum.	Minimum.			
		Mean.	Maximum.	Minimum.					
1915.							*Inches.*	*Inches.*	*Miles.*
August	16–20	61	80	61	84	57	0. 03	0. 790	3. 2
Do	21–25	70	81	60	84	57	. 46	1. 018	4. 4
Do	25–31	63	77	50	85	40	.....	1. 313	4. 7
September	1– 5	68	83	55	87	51	. 82	1. 424	7. 4
Do	6–10	69	81	56	91	54	.....	1. 029	6. 3
Do	11–15	71	84	60	97	53	T.	. 983	7. 2
Do	16–20	69	82	55	87	39	. 20	1. 072	5. 2
Do	21–25	66	76	58	84	50	1. 00	. 864	18. 2
Do	25–30	56	67	48	78	44	. 25	. 665	4. 4

During the growing months of May, June, July, August, and September in 1914 the rainfall amounted to only 6.42 inches, while during the same months in 1915 it amounted to 17.88 inches. Table II gives the number of inches of rainfall for each month during 1914 and 1915.

TABLE II.—*Rainfall (in inches) at Garden City, Kans., in 1914 and 1915*

Month.	Year.		Month.	Year.	
	1914	1915		1914	1915
January	None.	None.	July	0. 56	1. 66
February	Trace.	2. 53	August	. 64	6. 60
March	Trace.	. 18	September	. 15	2. 27
April	1. 74	2. 67	October	1. 48	1. 79
May	3. 63	4. 39	November	Trace.	. 12
June	1. 44	2. 96	December	. 06	1. 6

The summer of 1914 was much warmer than that of 1915, and the evaporation for each of the five growing months, with but one exception, was appreciably lower in the latter year than in the former. The evaporation from a free water surface for each month of the growing season is given in Table III.

TABLE III.—*Evaporation (in inches) at Garden City, Kans., for the growing months of 1914 and 1915*

Month.	Year.		Month.	Year.	
	1914	1915		1914	1915
May	7. 046	7. 593	August	10. 010	5. 920
June	9. 942	7. 699	September	9. 366	6. 037
July	9. 403	9. 258			

The evaporation during 5-day periods for the two growing seasons is shown graphically in figure 1.

GENERAL OUTLINE OF THE WORK

The experiments herein reported were conducted with Pride of Saline corn, Blackhull kafir, and Dwarf milo. The plants were grown both in the field and in large galvanized-iron cans. The investigations with the plants in the field included (1) the isolation of the root systems of

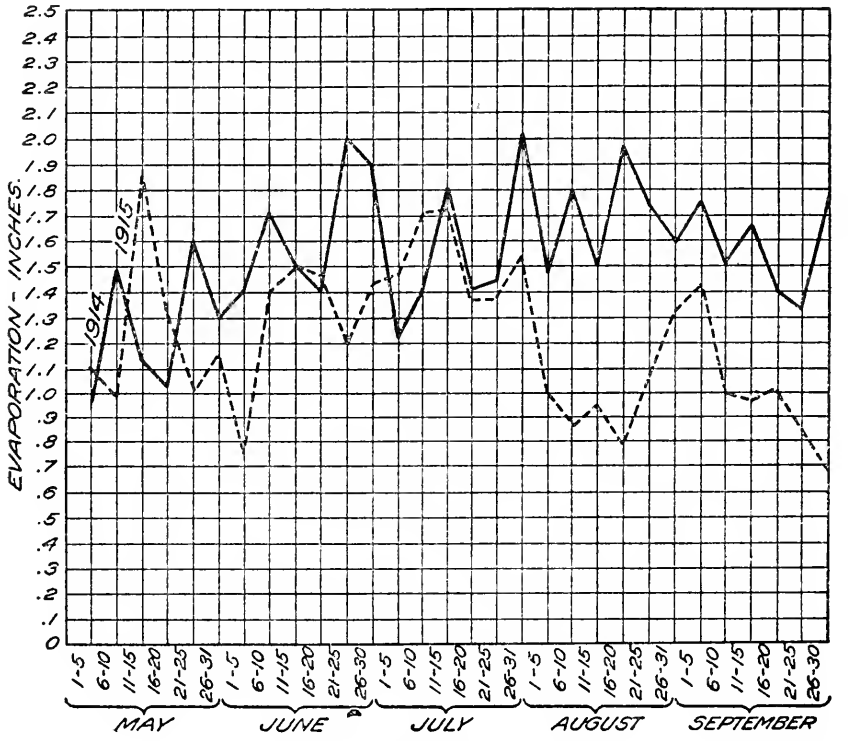


FIG. 1.—Evaporation from a free water surface (tank) at Garden City, Kans., during the growing seasons of 1914 and 1915.

corn, Blackhull kafir, and Dwarf milo at three stages of their growth; (2) a study of these root systems in relation to their general extent, as well as the number of their primary and secondary roots; (3) a comparative study of the leaf and sheath areas of these three plants at four periods of their growth; (4) a study of the soil-moisture content and the depth of root penetration.

The plants grown in the large iron containers furnished the material for a study of the relative dry weights of the roots and aerial portions of corn, Blackhull kafir, and Dwarf milo.

CULTURAL METHODS

The soil in which the plants were grown is known as a sandy loam of the Richfield series and shows very little difference in its texture in the upper 10 feet. Tables VIII and IX give the moisture equivalent and the wilting coefficient (1, p. 56–73) [1] for the soil at each foot to a depth of 10 feet on the plots which were used in 1914 and 1915, respectively.

The plants were grown on plots which had been in Dwarf milo the previous season. The land was plowed in the fall to a depth of 6 inches and then irrigated with approximately 8 to 10 inches of water or until the soil was saturated to a depth of from 3 to 4 feet. It received no further attention until spring, when it received several shallow cultivations, was then harrowed, and before planting was leveled with a float.

In order that the plants might be under the same conditions, the corn, kafir, and milo were planted in alternate rows on the same plots. On May 23, 1914, and on May 26, 1915, the crops were surface-planted in rows 44 inches apart. After the plants were a few inches in height the corn in the rows was thinned to a distance of 1½ to 2 feet between the plants, Blackhull kafir from 1 to 1½ feet, and the Dwarf milo from 8 inches to 1 foot. The plants were kept free from suckers at all times during the growing season. The plots were scraped with a hoe as often as was necessary to keep them free from weeds, but no other cultivation was given. After the fall irrigation the plots received no water other than that from the rainfall.

The relative weights of the root systems and aerial portions of the corn, Blackhull kafir, and Dwarf milo were obtained from plants grown in large sealed metal containers. These cans were made of 22-gauge galvanized iron and were 24 inches in height with a diameter of about 15 inches; and in this experiment each can contained from 100 to 110 kgm. of soil. The surface foot of the field soil was worked through a ¼-inch mesh screen and then thoroughly tamped in the cans. This soil was in good tilth, and for both seasons had a moisture content of 20 to 21 per cent (dry basis). This moisture content was kept approximately constant during the growing season by weighing the cans every 48 hours and then replacing the water that had been lost by the method used by Briggs and Shantz (2) in their work on the water requirement of plants. Different numbers of plants were grown in each can, as will be shown in the tables that record the data for this part of the work.

ISOLATION OF ROOT SYSTEMS IN THE FIELD

The root systems of plants growing under field conditions were isolated by a modification of the method devised by King (5).[2] This method, stated briefly, consists of the isolation of a prism of soil con-

[1] Reference is made by number to "Literature cited," p. 331.

[2] The work of other investigators concerning the development of root systems will be mentioned in this article only in so far as it is necessary to give a clear discussion of the experiments reported. The studies that have been made by other investigators on the development of the root systems of agricultural plants have been reviewed in detail elsewhere by the writer.

taining the plants whose root systems are desired and then placing over this block of earth a wire cage of such a shape and size as to fit closely to the vertical sides of the block. Numerous small wires are then run through the prism of earth and fastened to each side of the cage. The plants are fastened to the cage at the surface of the soil and the roots washed free from dirt by means of a stream of water. When the earth is washed away, the main roots remain suspended on the cross wires in the same position that they occupied in the soil.

This method is open to criticism, first, because in order to use it with any degree of satisfaction the prism of soil must be limited to about 18 inches in thickness, and on this account one obtains only a section of the root system. Furthermore, the main roots of the plant may not be in the prism of soil which has been isolated; therefore, when the soil is washed away, only a poor representation of the root system is obtained. Finally, although the primary roots of the plant remain on the wires in the same position that they occupied in the soil, it is impossible to retain all the finer roots in their normal position. No method has been devised, so far as is known to the writer, whereby the root systems of mature plants growing in the field under natural conditions can be isolated intact. The method of Rotmistrov (6) for obtaining complete root systems is open to criticism because root systems certainly would not develop normally in so small a volume of soil. For a comparative study of the general nature of the root systems of plants, growing under field conditions, the modified method of King as used in these experiments seems to be the least objectionable.

In the work reported in this paper, sections of the root systems were obtained crosswise of the rows. The prisms of soil varied from 15 to 18 inches in thickness and were isolated by digging a trench 2½ feet wide around them. After the isolation of a prism of soil, a wooden framework of light material was fitted snugly over it. Ordinary wire fencing with a 4- to 6-inch rectangular mesh was placed on two sides of the framework (Pl. XXXVIII, fig. 1, 2). This was found to be much more satisfactory than the poultry netting used by King and Ten Eyck, since the small mesh of such netting made it impossible to photograph the root systems with any degree of satisfaction after they had been isolated. The plant stubs and root crowns were held in place by wiring them to narrow strips of inch boards which were placed crosswise of the soil block at the surface of the soil and nailed to both sides of the framework of the cage. This method is much more convenient and simple than the one used by King (5) and Ten Eyck (9, 10, 11). In order to hold the plants in place, these investigators removed the upper portion of the soil surrounding the crown of the plant, and replaced it by a plaster of Paris cast.

For cross wires, ordinary broom wire was found to be the most satisfactory. Owing to the compactness of the soil, a ¼-inch iron rod pointed

at one end and provided with a wooden handle at the other was employed to make a passage through the soil block for the cross wires (Pl. XXXVIII, fig. 2). In the upper 2 feet of soil the cross wires were pushed through the block of soil at intervals of 3 to 4 inches on both the vertical and horizontal wires of the cage, while below that depth they were placed at the intersections only of the vertical and horizontal wires. In the isolation of the root systems of two mature plants, between 200 and 250 cross wires were pushed through the soil prism.

Several methods of washing the soil away from the roots were tried, but the following was found the most desirable: The trench around the block of soil was partially filled with water from an irrigation ditch near by; and then by means of a pitcher pump connected with a ¾-inch pipe of convenient length the water was pumped into a piece of galvanized-iron eaves trough and allowed to flow gently on the prism of soil (Pl. XXXVIII, fig. 3). In this manner the same water could be used over and over again. As soon as any of the larger roots were exposed they were carefully tied to the cross wires so that they would not be moved from their original position by the further washing. When the dirt that had been washed from the soil prism had filled the trenches to the surface of the water, the washing was discontinued and the water allowed to soak away. The soil that had been washed into the trenches was then removed, the trench again partially filled with water, and the washing continued. This routine, especially in working with mature plants, had to be repeated several times. After the soil had been washed from all the roots, the cages containing them were taken up, the unused cross wires removed and the root systems studied and photographed.

ISOLATION OF THE ROOT SYSTEMS FROM LARGE VESSELS

The following method was used in the isolation of the root systems of the plants that were grown in large galvanized-iron cans:

As soon as the aerial portions of the plants were harvested, the soil contained in the can was emptied upon a cleared space; and all the larger roots were removed from the soil by carefully working it over, a handful at a time. In order to separate the soil from the root particles still remaining in it, as much of the soil as possible was shaken through a sieve with a $\frac{1}{16}$-inch mesh. In this manner all the finer root portions, together with the larger soil particles, remained upon the screen. The root remnants and the soil particles on the sieve were then placed in a vessel and covered with a large excess of water, which was stirred vigorously until all the lumps of soil had disintegrated. All the root remnants floated to the surface of the water, and as soon as the soil in the vessel had settled, they were removed by pouring the water upon the fine sieve. All the roots which were obtained from each can were placed upon the fine screen and washed carefully a number of times until, so

far as could be seen, they were free from sand particles. The roots were then dried in a hot-air oven at 105° C. and their dry weight obtained.

DETERMINATION OF THE LEAF AREA

For obtaining the leaf and sheath areas five representative plants of the corn, kafir, and milo, respectively, were selected at the desired stage of growth. Their leaves and sheaths were cut into convenient pieces, and the outlines of these portions were carefully traced with a hard lead pencil on ordinary unruled paper. The outlines thus obtained were traced with a polar planimeter and the inclosed area calculated. In dealing with that portion of the leaf which was not yet fully unfolded, care was taken that the measurements included only that surface of the unfolding leaf that was exposed to the air.

GENERAL DISCUSSION OF EXPERIMENTAL DATA

EXTENT OF THE ROOT SYSTEMS

The root systems of corn, kafir, and milo growing in the field were isolated at four stages of growth in 1914 and at three stages in 1915. A summary of the general extent of the root systems of these plants is given in Table IV.

TABLE IV.—*General summary of the root systems isolated during the summers of 1914 and 1915 at Garden City, Kans.*

Date.	Crop.	Height of plants.	Greatest depth of root penetration.	Greatest lateral extent of roots.	Greatest length of a single root.	General remarks.
		Ft. in.	*Ft. in.*	*Ft. in.*	*Ft. in.*	
1914.						
June 24	Corn, Pride of Saline....	1 6	1 4	2 9	3 3	4 fully unfolded and 4 partially unfolded leaves.
	Kafir, Blackhull.....	1 0	1 6	3 0	3 5	Do.
	Milo, Dwarf.....	1 0	1 6	3 0	3 7	Do.
July 17	Corn, Pride of Saline....	3 6	3 0	3 6	3 8	8 fully and 6 partially unfolded leaves.
	Kafir, Blackhull.....	2 6	2 6	4 0	5 0	6 fully and 4 partially unfolded leaves.
	Milo, Dwarf.....	2 6	2 9	3 0	4 2	"Rooting."
Aug. 1	Corn, Pride of Saline....	5 6	4 0	2 6	4 6	"Shooting."
	Kafir, Blackhull.....	4 0	4 0	3 6	5 8	Heading.
	Milo, Dwarf.....	3 0	4 0	3 6	5 6	Seed in milk stage.
Aug. 25	Corn, Pride of Saline....	6 0	6 0	3 0	7 0	End of vegetative growth, grains glazed.
	Kafir, Blackhull.....	5 0	6 0	3 10	8 2	Seed in milk.
	Milo, Dwarf.....	3 0	6 0	3 6	7 6	Seed fully ripe.
1915.						
July 10	Corn, Pride of Saline....	1 6	1 3	2 10	3 0	4 fully and 4 partially unfolded leaves.
	Kafir, Blackhull.....	1 0	1 6	2 0	2 3	Do.
	Milo, Dwarf.....	1 0	2 0	2 0	2 6	Do.
30	Corn, Pride of Saline....	5 0	4 6	3 8	6 0	Tassel peeping.
	Kafir, Blackhull.....	3 6	4 6	3 8	6 3	7 fully unfolded and 5 partially unfolded leaves.
	Milo, Dwarf.....	3 0	4 6	3 8	6 0	Blooming.
Sept. 3	Corn, Pride of Saline....	7 0	6 0	3 8	7 0	Early milk stage.
	Kafir, Blackhull.....	6 0	6 0	3 8	8 8	Blooming.
	Milo, Dwarf.....	3 6	6 0	3 8	6 8	Seed in milk stage.

Stage I.—At this period of growth, the plants of Dwarf milo and Blackhull kafir had reached a height of 1 foot and had four fully and four partially unfolded leaves, while the corn plants with the same number of leaves had a height of 1 foot 6 inches. In 1914 the plants reached this stage on June 24, four weeks from the time of planting the seed; but in 1915, owing to cool weather, they did not reach this stage until July 10, six weeks after seeding (Pl. XLIII, fig. 1).

In 1914 the greatest depth reached by the root system of the corn plant at this stage was 1 foot 4 inches, while the greatest depth of the kafir and milo roots was 1 foot 6 inches. At this time the roots of the corn extended horizontally to a distance of 2 feet 9 inches, while in the same direction the roots of both kafir and milo extended 3 feet (Pl. XXXIX, fig. 3). The depth of root penetration for corn and kafir at this stage was practically the same in 1915 as in 1914, but Dwarf milo exceeded the depth reached the previous year by 6 inches. The maximum lateral extent of the corn roots was the same as in 1914, but it was 1 foot less for the kafir and milo (Pl. XXXIX, fig. 2, 4).

At this time the differences exhibited by these three plants in their method of rooting were very slight. The number of primary roots which penetrated to a depth of a foot was between 12 and 15 for each plant, but more of the first primary roots of the corn took a horizontal direction than did those of the kafir and milo. On this account more of the primary roots of the latter penetrated to the maximum depth than did those of the corn plant. The secondary roots of all the plants grew both upward and downward from the primary roots, so that at this stage the upper foot of soil was filled with a network of roots to within ¼ inch of the surface.

Stage II.—The root systems at this period of growth were isolated only in 1914. At this time the corn plants had reached a height of 3½ feet and had 8 fully and 6 partially unfolded leaves, while Blackhull kafir, with approximately the same number of leaves, had a height of 2½ feet. The Dwarf milo plants had from 9 to 10 fully unfolded leaves, including the "boot" leaf, and stood 2½ feet high. The plants reached this stage on July 17, seven weeks from the time of planting (Pl. XLIV, fig. 1).

The greatest depth reached by the corn roots at this time was 3 feet, while the maximum depth for Blackhull kafir and Dwarf milo was 2 feet 6 inches and 2 feet 9 inches, respectively. The greatest lateral extent reached by the roots of corn and Dwarf milo at this period was 3 feet, while the roots of standard kafir extended horizontally for a distance of 4 feet. The tendency of the first primary roots of the corn to take a more horizontal direction than those of the sorghums is well shown at this stage (Pl. XXXIX, fig. 1).

It was found that the later roots of the corn take the same general direction as do those of Blackhull kafir and Dwarf milo, and that the maximum depth of root penertation is practically the same for all three plants.

Stage III.—In 1914 the roots of the three plants were isolated about the first of August, 10 weeks from the time of planting. The corn at this stage was shooting and had a height of 5½ feet, while Blackhull kafir was heading and stood 4 feet high. The seed of the Dwarf milo was in the milk stage, and the plant had reached a height of 3 feet.

The greatest depth of root penetration at this stage was 4 feet for all the plants. The maximum lateral extent of the roots of corn was 2½ feet, while the roots of both Blackhull kafir and Dwarf milo showed a maximum horizontal extent of 3½ feet (Pl. XL, fig. 2).

The roots at this stage were isolated on July 17, 1915, when the plants had reached the same age at which they were examined the previous year. The corn at this date stood 5 feet high, and the tassel was just beginning to show. Blackhull kafir stood 3½ feet high and had seven fully and five partially unfolded leaves. The Dwarf milo was blooming and had a height of 3 feet.

The maximum depth and lateral extent of the roots at this stage was found to be approximately the same for all three plants. The greatest depth of the roots was 4½ feet, while the greatest extent in a horizontal direction was approximately 3⅔ feet.

Stage IV.—The root systems at this stage were isolated on August 25, 1914, when the plants were 13 weeks old. The corn had reached a height of 6 feet and the grain was in a glazed condition. The seed of Blackhull kafir was in the milk stage and the plants which stood 5 feet high had reached their maximum vegetative growth. The seed of the Dwarf milo was fully ripe, and the plants had made little if any growth since the previous stage (Pl. XLIV, fig. 2).

The roots of all three plants were found to reach a maximum depth of 6 feet, while the greatest lateral extent for all three was between 3 and 4 feet (Pl. XL, fig. 1).

In 1915 the plants had not reached their full vegetative growth until September 3, and even at that date they were not nearly as mature as those examined at the same age in 1914. The corn was 7 feet high, and the grain was in the early milk stage. Blackhull kafir was in bloom and had a height of 6 feet, while the grain of the Dwarf milo was in the milk stage and the plants stood 3½ feet high.

The maximum depth of the root systems was 6 feet for each plant, while while the maximum extent horizontally for each was 3⅔ feet (Pl. XLI, fig. 1, 2).

Both the primary and secondary roots of Dwarf milo and Blackhull kafir at all stages of growth were more fibrous than those of the corn. The length of the secondary roots was found to be approximately the same for the three plants at any given stage of growth. The secondary roots of kafir and Dwarf milo broke so easily in the washing process that it was almost impossible to obtain them intact from the soil which was used in this experiment (Pl. XLII, fig. 1, 2).

NUMBER OF SECONDARY ROOTS

It has been shown in the foregoing discussion of the isolation of the root systems of corn, Blackhull kafir, and Dwarf milo at the various periods of growth, that no marked differences were to be observed between these plants in regard to the number and general extent of their primary roots. It was thought advisable on this account to make a study of the number of secondary roots possessed by the three plants at different stages of growth.

After the isolated root systems had been studied and photographed the primary roots of each plant were cut into inch lengths and the number of the secondary roots originating from each unit of length was determined under a dissecting microscope. The results of this investigation for all the stages of root growth examined in 1914 and 1915 are shown in Table V. It was found from 321 observations of the roots of the corn, 311 of Dwarf milo and 210 of Blackhull kafir that the number of secondary roots per unit of length of primary root was approximately twice as great for the two sorghums as for the corn. This fact stands out strikingly not only for each year but for all the different stages of the development of the root systems (Pl. XLII, fig. 1, 2).

TABLE V.—*Number of secondary roots per unit of length of primary roots of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.*

Year and crop.	Stage of growth (height of plants in feet).	Number of observations.	Average number of roots per inch.	Average number of roots per centimeter.
1914.				
Corn, Pride of Saline	1½	33	15	6
	3½	37	17	7
	6	57	12	5
	6	32	11	4
Milo, Dwarf	1	21	25	10
	2½	54	29	12
	3	72	26	10
Kafir, Blackhull	2½	40	31	12
	5	60	26	10
1915.				
Corn, Pride of Saline	1½	50	16	6
	5	65	12	5
	7	47	12	5
Milo, Dwarf	1	24	23	9
	3	70	30	12
	3½	70	25	10
Kafir, Blackhull	1	40	20	8
	6	70	20	8

WEIGHT OF THE ROOTS AND AERIAL PORTIONS OF THE PLANTS

A comparative study was made of the dry weight of the aerial parts and roots of corn, Blackhull kafir, and Dwarf milo in 1914, and for these three plants and Dwarf Blackhull kafir in 1915. The root systems that

were isolated for this study were obtained from mature plants which were grown primarily for transpiration studies in the large metal cans previously described. The plants made a vigorous growth and compared very favorably in every way with the plants that were grown under field conditions.

Three corn plants were grown in each can during both seasons. In 1914 the corn reached a height of 5 feet, and in 1915 it stood 6 feet high, but no grain was produced in either season. In 1914 six Dwarf milo plants were grown in each can, but in 1915 the number of plants was reduced to three to each can. Six Blackhull kafir plants were grown to each can in 1914 and three plants to each can in 1915.

The Dwarf milo reached a height of 3 feet in 1914, while in 1915 it stood 4½ feet high. The Blackhull kafir plants attained a height of 5 feet in 1914, but in 1915 they reached a height of 6 feet. Dwarf Blackhull kafir was planted during the season of 1915 only, and three plants were grown in each can. These plants reached a height of 4½ feet. The results for the two seasons are shown in Table VI.

TABLE VI.—*Relative weight of the roots and aerial portions of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.*

1914

Crop and can No.	Number of plants.	Weight of stem, leaves, and grain.	Weight of stem and leaves.	Weight of roots.	Ratio of the weight of stem, leaves, and grain to weight of the roots.	Ratio of the weight of the stem and leaves to the weight of the roots.
Milo, Dwarf:		*Gm.*	*Gm.*	*Gm.*		
1	6	187.3	115.5	11.7	16	9.8
2	6	161.5	121.1	10.7	15	11.3
3	6	173.9	128.7	12.9	13.4	9.9
4	6	184.4	105.1	12.0	15.3	8.7
5	6	161.7	102.9	12.0	13.4	8.5
6	6	159.7	91.2	9.5	16.8	9.6
Average ratio					15.0	9.6
Kafir, Blackhull:						
7	4	217.9	163.4	16.5	13.2	9.9
8	5	234.1	167.4	12.9	18.1	12.9
9	5	212.6	157.1	14.2	14.9	11.0
10	6	219.5	159.0	13.8	15.9	11.5
11	4	175.6	123.6	10.9	16.1	11.3
12	6	257.3	180.0	20.8	12.3	8.8
Average ratio					15.0	10.9
Corn, Pride of Saline:						
13	3		150.6	13.7		10.7
14	3		153.9	15.9		9.6
15	3		131.4	15.6		8.4
16	3		163.7	16.4		9.9
Average ratio						9.6

TABLE VI.—*Relative weight of the roots and aerial portions of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.*—Continued

1915

Crop and can No.	Number of plants.	Weight of stem, leaves, and grain.	Weight of stem and leaves.	Weight of roots.	Ratio of the weight of stem, leaves, and grain to weight of the roots.	Ratio of the weight of the stem and leaves to the weight of the roots.
Milo, Dwarf:		*Gm.*	*Gm.*	*Gm.*		
2	3	214.6	111.5	13.5	15.8	8.2
3	3	226.4	111.8	12.7	17.8	8.8
5	3	231.4	125.8	14.0	16.5	8.9
6	3	223.3	121.3	22.4	[a] (9.9)	[a] (5.4)
7	3	233.3	123.7	15.0	15.5	8.2
8	3	217.6	110.0	14.0	15.5	7.8
11	3	230.5	115.8	16.8	13.7	6.8
12	3	225.8	117.5	15.0	15.0	7.8
Average ratio					15.7	8.0
Kafir, Dwarf Blackhull:						
13	3	249.7	142.7	16.0	15.6	8.9
14	3	221.8	133.4	13.6	16.3	9.8
15	3	257.8	137.9	15.4	16.7	8.9
16	2	168.8	97.1	10.4	16.2	9.3
17	3	230.2	135.1	16.9	13.6	8.0
Average ratio					15.7	8.9
Kafir, Blackhull:						
18	3	341.7	215.0	19.0	17.9	11.3
21	2	219.3	147.2	14.6	15.0	10.0
53	3	299.7	207.3	25.0	11.9	8.2
54	3	287	206.3	23.5	12.2	8.7
55	3	310.3	213.1	14.7	[a] (21.1)	[a] (14.4)
56	3	342.8	253.2	21.0	16.3	12.0
57	3	333.8	219.5	20.1	16.6	10.9
58	3	354.2	244.6	14.7	[a] (24.0)	[a] (16.6)
Average ratio					14.9	10.1
Corn, Pride of Saline:						
24	3	.....	205.6	30.5	.....	6.7
25	3	.....	252.5	33.1	.....	7.6
26	3	.....	234.4	26.0	.....	9.0
27	3	.....	202.4	28.2	.....	7.1
28	3	.....	211.2	33.1	.....	6.3
29	3	.....	228.3	31.7	.....	7.2
42	3	.....	239.7	24.7	.....	9.7
43	3	.....	249.3	28.1	.....	8.8
Average ratio						7.8

[a] Not included in the average.

The root systems of Dwarf milo and Blackhull kafir were isolated from six cans in 1914 and from eight cans in 1915. The average ratio of the dry weight of the grain and of the stem and leaves of Dwarf milo to the dry weight of the roots was as 15 to 1 in 1914 and as 15.7 to 1 in 1915.

The dry weight of the stem and leaves was 9.6 times the weight of the roots in 1914, and 8 times their weight in 1915. In 1914 the dry weight of the grain, stem, and leaves of Blackhull kafir was 15 times that of the roots, while the ratio of the dry weight of the stem and leaves to the dry weight of the roots was as 10.9 to 1. The average ratio of the weight of all the aerial parts to the root weight in 1915 was as 14.9 to 1, while the weight of the stem and leaves was 10.1 times that of the roots. In 1914 root systems of corn were obtained from 4 cans and from 10 cans in 1915. The average ratio of the weight of the stem and leaves to the weight of the roots was 9.6 in 1914 and 7.8 in 1915. The roots of Dwarf Blackhull kafir were isolated from five cans in 1915. The weight of all the aerial parts was 15.7 times that of the roots, while the ratio of the weight of the stem and leaves to the weight of the roots was 8.9 to 1.

For the purpose of comparison the results obtained by various investigators for the relative weights of the tops and roots of plants are given here. It must be borne in mind, however, that the relative weights of the roots and aerial portions of plants vary according to the conditions under which they are grown. It has been shown (4, 8, 12) that, among other factors, the soil-moisture content and the amount of available plant nutrients are important in determining the ratio of the weight of the tops of plants to their root weight. Hellriegel (3) found the ratio of the aerial portions of mature barley and oat plants to the weight of their roots to be 11.6 to 1, and 6.6 to 1, respectively. Schulze (7) reports the ratio of the weight of the aerial portions to the weight of the roots to be 10.8, 13.5, and 11.1, respectively, for mature wheat, barley, and oat plants. King (5) found the weight of the aerial part of mature corn to be 7 times that of the root weight, while Kiesselbach (4) found the ratio of the weight of the tops to the root weight to be 8.5 for corn plants grown in a soil with a water content of 98 per cent and 5.2 for plants growing in a soil with a water content of 20 per cent.

SOIL-MOISTURE CONTENT AND THE DEPTH OF ROOT PENETRATION

In order to be able more exactly to define the conditions under which the plants used for root examinations were grown, soil samples for moisture determinations were taken at intervals of from 10 to 14 days from the plots upon which the corn, standard kafir, and Dwarf milo grew. Since the moisture content of the soil was determined a few days before or a few days after the isolation of the various root systems, it was possible to compare the depth of the penetration of the roots with the depth of the moisture depletion of the soil.

The results of these observations are given in Table VII. The moisture content of the soil for each foot to a depth of 10 feet is shown for several periods of the two growing seasons. The depth of the root penetration was determined from the root systems isolated at the various stages

which have already been described. The moisture equivalent, together with the wilting coefficient obtained from it by the formula of Briggs and Shantz (1, p. 56–73) for each foot of soil, is also included therein.

TABLE VII.—*Soil-moisture content and depth of root penetration of corn, kafir, and milo in 1914 and 1915 at Garden City, Kans.*

Date.	Percentage of moisture at a depth of—										Greatest depth of roots.		
	1 foot.	2 feet.	3 feet.	4 feet.	5 feet.	6 feet.	7 feet.	8 feet.	9 feet.	10 feet.	Corn.	Kafir.	Milo.
1914.											*Feet.*	*Feet.*	*Feet.*
June 5	22.9	22.5	21.1	22.7	23.5	21.2	16.1	17.0	16.4	14.5	.....	.....	.....
July 2	14.6	20.2	21.2	22.8	22.1	21.8	.....	.....	.....	.....	1	1½	1½
10	11.8	17.1	19.5	23.6	24.6	21.4	.....	.....	.....	.....	.....	.....	.....
21	10.6	13.3	14.5	19.4	22.8	21.7	.....	.....	.....	.....	3	2¾	3
29	8.7	13.1	13.5	16.8	21.4	21.0	.....	.....	.....	.....	4	4	4
Aug. 9	9.4	13.5	13.2	14.5	20.3	19.5	.....	.....	.....	.....	.....	.....	.....
22	8.4	13.4	12.4	14.4	19.2	19.7	.....	.....	.....	.....	6	6	6
Sept. 6	7.7	12.2	11.4	12.9	15.6	19.1	18.2	18.9	16.7	13.0	6	6	6
Wilting coefficient of Briggs and Shantz	12.7	14.5	14.5	16.3	17.1	16.1	15.7	15.7	15.8	15.0	.....	.....	.....
Moisture equivalent	23.4	26.7	26.7	30.0	31.5	29.6	29.0	28.9	29.2	27.6	.....	.....	.....
1915.													
June 18	21.0	21.4	21.7	18.5	15.5	16.0	16.6	19.1	18.8	19.2	.....	.....	.....
29	20.3	20.8	20.8	17.9	16.8	16.7	17.1	19.3	18.4	19.7	.....	.....	.....
July 12	16.2	20.8	20.2	17.8	19.0	15.5	.....	.....	.....	.....	1¼	1¼	2
24	12.0	15.7	17.8	17.2	17.4	16.5	.....	.....	.....	.....	.....	.....	.....
Aug. 6	14.2	15.2	15.4	16.0	16.1	15.4	17.9	18.6	20.6	20.2	4½	4½	4½
16	21.0	20.7	17.7	17.2	16.4	15.5	18.0	19.0	19.5	20.4	.....	.....	.....
25	17.0	17.9	15.9	15.5	15.6	16.2	19.8	19.9	19.7	20.9	6	6	6
Wilting coefficient of Briggs and Shantz	13.3	14.1	14.9	13.6	13.4	11.9	12.4	12.1	11.1	13.0	.....	.....	.....
Moisture equivalent	24.4	25.9	27.5	25.1	24.6	21.9	22.8	22.3	20.5	23.9	.....	.....	.....

The season of 1914 was especially favorable for such an observation, since the rainfall for the last half of June amounted to only 0.44 inch, and for July and August 0.56 and 0.64 inch, respectively. This amount of rainfall, a little over 1½ inches for the 2½ months, came at 12 different periods, so that with the exception of the first foot of soil no influence was exerted by the rainfall upon the original soil-moisture content. The season of 1915 was not so favorable for an observation of this kind, but the results, while not so striking as those of 1914, show the same facts. It should be borne in mind in studying Table VII that in 1914 the soil samples which were taken on July 2 and 21 were procured from five to six days after the isolation of the root systems whose depths are recorded for that date. Furthermore, in 1915 the samples for July 12 and August 6 were taken two and six days, respectively, after the recorded depths of the root systems.

The results of these experiments for both seasons seem to show that there was little if any depletion of the soil moisture below the depth to which the roots penetrated.

LEAF AND SHEATH AREAS[1]

The leaf and sheath areas of corn, Blackhull kafir, and Dwarf milo were determined at four stages of growth in 1914. The results of these measurements are shown in Table VIII. Figures 2 and 3 represent these areas graphically.

TABLE VIII.—*Dry weight, leaf and sheath areas of corn, kafir, and milo at different stages of growth in 1914 at Garden City, Kans.*

Plant and period of growth.	Plant No.	Dry weight of leaves and stems.	Leaf area.		Sheath area.	
		Gm.	*Sq. in.*	*Sq. cm.*	*Sq. in.*	*Sq. cm.*
STAGE I.						
Corn, Pride of Saline, June 24, 1914, one month from time of planting.	1	12.3	272.2	1,756.1	24.1	155.4
	2	11.2	230.6	1,487.5	15.0	96.7
	3	15.2	285.7	1,842.6	23.9	154.4
	4	9.6	205.8	1,327.6	12.8	82.6
	5	9.2	210.2	1,355.7	14.5	93.9
Average		11.5	241.0	1,553.9	18.0	116.6
Kafir, Blackhull, June 24, 1914, one month from time of planting.	1	9.0	141.3	911.7	15.9	102.8
	2	11.4	191.9	1,238.1	15.7	100.9
	3	7.5	145.0	935.2	10.2	65.5
	4	7.5	138.0	890.1	9.8	63.2
	5	5.4	116.8	753.4	8.4	54.2
Average		8.1	146.7	945.7	12.0	77.3
Milo, Dwarf, June 24, 1914, one month from time of planting.	1	6.1	140.0	903.2	9.6	62.2
	2	6.6	138.3	892.0	10.5	67.8
	3	7.0	149.0	961.3	9.7	62.6
	4	7.3	155.6	1,003.8	10.4	66.8
	5	5.5	122.1	787.3	9.1	58.8
Average		6.5	141.0	909.5	9.8	63.6
STAGE II.						
Corn, Pride of Saline, July 7, 1914, six weeks from time of planting.	1	51.1	902.7	5,822.5	71.2	459.0
	2	49.7	842.3	5,433.1	67.8	437.3
	3	49.5	828.6	5,344.2	81.2	523.5
	4	50.0	877.3	5,658.6	54.0	348.3
	5	54.7	754.3	4,865.5	67.3	434.2
Average		51.0	841.0	5,424.7	68.3	440.4
Kafir, Blackhull, July 7, 1914, six weeks from time of planting	1	27.8	372.3	2,401.6	26.3	169.6
	2	31.4	438.0	2,825.4	35.8	231.2
	3	31.0	484.0	3,122.1	42.7	275.7
	4	29.9	420.7	2,713.8	41.0	264.4
	5	27.5	338.2	2,181.4	29.3	189.3
Average		29.5	408.6	2,635.8	35.0	226.0
Milo, Dwarf, July 7, 1914, six weeks from time of planting	1	22.8	402.4	2,595.7	37.4	241.5
	2	28.3	456.4	2,943.6	37.0	238.6
	3	23.4	363.9	2,347.1	32.2	207.4
	4	24.5	343.2	2,214.0	31.9	205.4
	5	26.8	397.3	2,562.6	32.7	211.2
Average		25.1	392.6	2,532.5	34.2	220.8
STAGE III.						
Corn, Pride of Saline, July 21, 1914, eight weeks after planting	1	114.6	1,231.8	7,945.2	127.7	822.2
	2	137.2	1,423.7	9,182.9	127.2	820.7
	3	149.2	1,378.2	8,889.3	138.8	895.5
	4	140.1	1,266.6	8,169.8	124.8	805.2
	5	102.8	1,366.6	8,811.6	92.7	598.4
Average		128.7	1,333.4	8,600.4	122.3	788.8
Kafir, Blackhull, July 21, 1914, eight weeks from time of planting	1	70.1	987.1	6,367.2	69.1	445.8
	2	75.9	965.7	6,228.7	57.8	334.5
	3	60.7	829.3	5,349.0	50.4	325.0
	4	68.3	893.9	5,766.2	82.8	534.4
	5	67.8	689.2	4,445.4	42.9	276.9
Average		68.5	873.0	5,631.3	59.4	383.3

[1] In this paper the term "leaf area" means the surface inclosed by the margins of the leaves. The total leaf surface exposed to the air therefore would be twice the leaf area.

TABLE VIII.—*Dry weight, leaf and sheath areas of corn, kafir, and milo at different stages of growth in 1914 at Garden City, Kans.*—Continued

Plant and period of growth.	Plant No.	Day weight of leaves and stems.	Leaf area.		Sheath area.	
		Gm.	*Sq. in.*	*Sq. cm.*	*Sq. in.*	*Sq. cm.*
STAGE III—continued.						
Milo, Dwarf, July 21, 1914, eight weeks from time of planting. Leaf growth completed.	1	48.6	606.4	3,911.4	73.0	471.2
	2	54.1	664.6	4,286.8	45.8	295.8
	3	49.8	588.5	3,796.2	44.6	287.8
	4	57.0	593.3	3,827.0	53.7	346.49
	5	47.7	572.5	3,693.0	40.5	260.9
Average		51.4	605.1	3,902.9	51.4	332.4
STAGE IV.						
Corn, Pride of Saline, Aug. 4, 1914, ten weeks from time of planting. Leaf growth completed	1	167.4	1,273.6	8,215.0	192.1	1,239.3
	2	197.1	1,630.7	10,517.7	269.4	1,737.9
	3	171.0	1,324.6	8,543.7	210.9	1,360.6
Average		178.5	1,409.6	9,092.1	224.1	1,445.9
Kafir, Blackhull, Aug. 4, 1914, ten weeks from time of planting. Leaf growth completed	1	84.5	784.4	5,059.3	83.7	540.2
	2	123.0	992.5	6,401.9	94.0	606.3
	3	113.9	917.0	5,914.9	103.5	667.9
	4	83.0	871.2	5,619.2	94.8	611.5
Average		101.1	891.3	5,748.8	93.2	606.5
Milo, Dwarf, Aug. 4, 1914, ten weeks from time of planting. Leaf growth completed at Stage III	1	70.6	.....	.....	85.9	554.1
	2	54.6	.....	.....	67.6	435.9
	3	79.2	.....	.....	102.1	658.5
	4	69.5	.....	.....	89.4	576.6
Average		66.2	605.1	3,902.9	86.2	556.2

SUMMARY

Plant and period of growth.	Height of plants.		Number of leaves.[1]	Dry weight of stem and leaves.	Leaf area.[2]		Sheath area.		Square centimeter of leaf area per gram of dry weight.
	Feet.	*Cm.*		*Gm.*	*Sq. in.*	*Cm.*	*Sq. in.*	*Sq. cm.*	
Stage I, June 24, 1914:									
Corn	1.5	45	4F 4P	11.5	241	1,553	18	116	135.0
Kafir	1.0	30	4F 4P	8.1	146	945	12	77	116.6
Milo	1.0	30	4F 4P	6.5	141	909	9	63	139.8
Stage II, July 7, 1914:									
Corn	2.5	75	6F 6P	51	841	5,244	68	440	102.8
Kafir	1.5	45	6F 4P	29.5	408	2,635	35	226	89.3
Milo	2.0	60	6F 3P	25.1	392	2,532	34	220	100.0
Stage III, July 21, 1914:									
Corn	4	120	9F 5P	128.7	1,333	8,600	122	788	66.8
Kafir	2.5	75	7F 3P	68.5	873	5,631	59	383	86.2
Milo	2.5	75	9	51.4	605	3,902	51	332	75.9
Stage IV, August 4, 1914:									
Corn	6	180	14–15	178.5	1,409	9,092	224	1,445	50.0
Kafir	4	120	12–14	151.1	891	5,748	93	626	55.8
Milo	3	90	9–10	66.2	605	3,902	86	556	58.9

[1] F= Leaves fully unfolded; P= Leaves partially unfolded. [2] Leaf surface equals twice these figures.

Stage I.—The plants reached this stage one month from the time of planting. Each plant showed four fully and four partially unfolded leaves. The Dwarf milo and Blackhull kafir plants had reached a height of 1 foot, while the corn plants stood 1½ feet high (Pl. XLIII, fig. 1). The leaf areas at this stage measured 1,553, 945, and 909 sq. cm. for corn, Blackhull kafir, and Dwarf milo, respectively, while the sheath areas of

these plants taken in the same order amounted to 116, 77, and 63 sq. cm. It is seen at this stage that the leaf area of corn was 1.7 times that of Dwarf milo and 1.64 times that of the Blackhull kafir.

Stage II.—The corn plants at this time had a height of 2½ feet and possessed six fully and six partially unfolded leaves. The Blackhull kafir measured 1½ feet in height and showed six fully and four partially unfolded leaves, while the Dwarf milo stood 2 feet high and had six fully and three partially unfolded leaves.

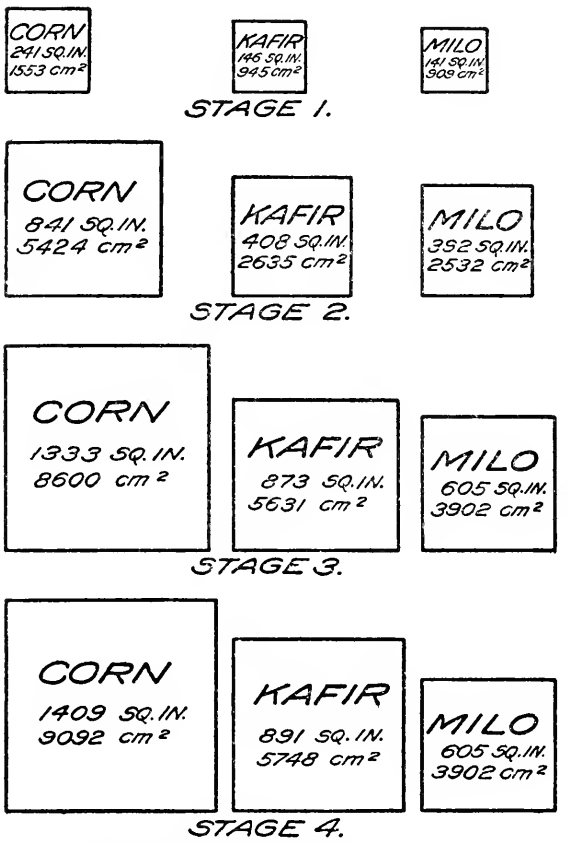


FIG. 2.—Comparison of the leaf areas of Pride of Saline corn, Blackhull kafir, and Dwarf milo at four stages of the growth of these plants during the season of 1914.

The plants reached this condition six weeks from the date of planting (Pl. XLIII, fig. 2). At this time the leaf area of the corn had increased to 5,424 sq. cm., while that of the Blackhull kafir and Dwarf milo measured 2,635 and 2,532 sq. cm., respectively, the leaf area of the corn having increased to twice that of the Dwarf milo or Blackhull kafir. The leaf area of the two sorghums increased at the same rate up to this stage. The sheaths of all three plants showed an area approximately three times larger than they did when examined in the first stage.

Stage III.—The plants at this period were 8 weeks old. The corn stood 4 feet high and had nine fully and five partially unfolded leaves. Blackhull kafir and Dwarf milo had each reached a height of 2½ feet. The former had seven fully and three partially unfolded leaves, while the latter was in the "booting stage" and possessed nine fully grown leaves (Pl. XLIV, fig. 1). The Dwarf milo at this stage had reached its full leaf development and showed a leaf area of 3,902 sq. cm. The leaf area of the corn plant was 2.2 times this, or 8,600 sq. cm. The leaf area of

Blackhull kafir had increased to 5,631 sq. cm. and was 1.44 times the leaf extent of the Dwarf milo. The sheath area of the corn, Blackhull kafir, and Dwarf milo measured 788, 383, and 332 sq. cm., respectively.

Stage IV.—The plants at this stage had reached an age of 10 weeks and had completed their leaf development. The corn plants had from 14 to 15 leaves and the standard kafir from 12 to 14 leaves. The corn plants were 6 feet high, the standard kafir 4 feet high, while the Dwarf milo had reached a height of 3 feet (Pl. XLIV, fig 2). The leaf area of the corn plant at maturity was 9,092 sq. cm., an area 2.3 times that of the mature Dwarf milo, and 1.53 times that of the Blackhull kafir. The sheath area of these three plants was 1,445, 605, and 556 sq. cm., respectively, for corn, Blackhull kafir, and Dwarf milo.

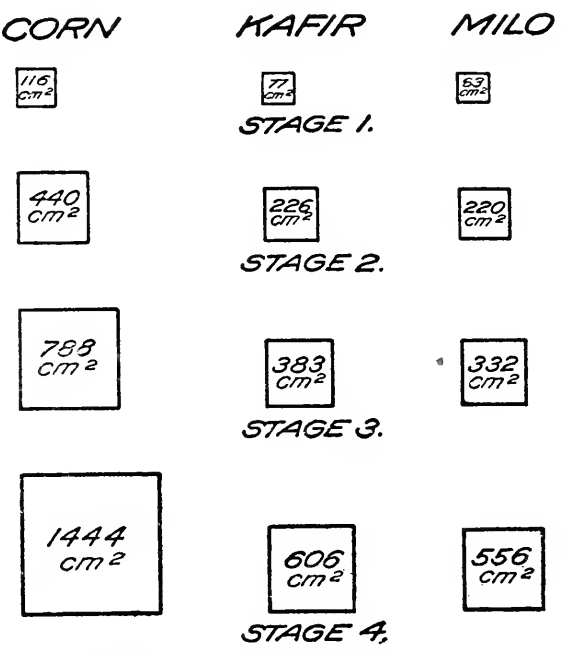


FIG. 3.—A graphic illustration of the sheath areas of Pride of Saline corn, Blackhull kafir, and Dwarf milo at four stages of the growth of these plants during the season of 1914.

SUMMARY

The root systems of Pride of Saline corn, Blackhull kafir, and Dwarf milo plants which were grown in alternate rows were isolated in the field at four stages of growth in 1914 and at three stages of growth in 1915. All told, the root systems of 33 plants were isolated and studied. It was found that for a given stage of growth each plant possessed the same number of primary roots and that the general extent of these roots in both a horizontal and vertical direction was the same for all three plants. The maximum depth of root penetration for mature Dwarf milo, Blackhull kafir, and corn was found to be 6 feet for both the years 1914 and 1915. It was found that Blackhull kafir and Dwarf milo possessed approximately twice as many secondary roots per unit of primary root as did the corn plant. This is true not only for both years but also for all stages of the root systems examined. Both primary and secondary roots of the sorghums were found to be more fibrous than those of the corn plant.

The relation of the weight of the dry matter of the aerial portions of mature plants to the weight of the roots was determined in 1914 for 36 Dwarf milo plants, 30 Blackhull kafir plants, and 12 corn plants. In 1915 the same determinations were made for 24 Dwarf milo plants, 14 Dwarf Blackhull kafir plants, 23 Blackhull kafir plants, and 24 corn plants.

The average ratio of the dry weight of the grain, stem, and leaves of standard kafir to the dry weight of the roots was found to be 15 and 14.9 for the years 1914 and 1915, respectively, while the dry weight of the stem and leaves of the same plant was on the average 10.9 times that of the root weight in 1914 and 10.1 times the root weight in 1915. The ratio of the dry weight of the stem, leaves, and grain of Dwarf milo to the weight of the roots was found to be as 15.7 to 1 in 1914, and as 15 to 1 in 1915, and the weight of the stem and leaves of the same plants was 9.6 and 8 times, respectively, the weight of the roots in 1914 and 1915. The weight of the stem and leaves of Pride of Saline corn was 9.6 times the root weight in 1914, while in 1915 the weight of the stem and leaves of the corn was 7.8 times the weight of the root system. The aerial parts of Dwarf Blackhull kafir examined in 1915 showed a weight 15.7 times that of the roots, while the weight of the stem and leaves amounted to 8.9 times the weight of the underground portion.

The results of the experiments for the two years in regard to the soil-moisture content and depth of root penetration seem to show that under the conditions of this experiment very little, if any, depletion of soil moisture took place below the depth of root penetration.

The average leaf areas of five representative plants of corn, Blackhull kafir, and Dwarf milo were obtained at stages when the plants were 4, 6, 8, and 10 weeks of age. The last stage examined showed that the plants had completed their full-leaf development. In all the stages of growth the corn plant was found to have the greatest leaf area. Taking the stages of growth in order, one finds that the leaf area of the corn plant was 1.7, 2.0, 2.2, and 2.3 times the leaf area of Dwarf milo and 1.6, 1.9, 1.5, and 1.5 times that of Blackhull kafir.

In comparing the plants of Dwarf milo, Blackhull kafir, and Pride of Saline corn, it will be seen that in all stages of their growth these two sorghum plants have a primary root system that is just as extensive as that of the corn plant. In addition, the Dwarf milo and Blackhull kafir possess twice as many secondary roots as the corn at any stage of its growth. The leaf area of the corn plant at all stages of its growth is approximately twice as great as that of the Dwarf milo and never less than 1.5 times that of Blackhull kafir.

It is apparent, therefore, that the Dwarf milo and Blackhull kafir plants would have the advantage over the corn plant under any climatic condition that would tend to bring about a loss of water from these plants.

The two sorghums have, in the first place, as compared to the corn plant, only one-half the leaf surface exposed for the evaporation of water; and in the second place they have a root system which, judging from the number of secondary roots, would be twice as efficient in the absorption of water from the soil.

LITERATURE CITED

(1) BRIGGS, L. J., and SHANTZ, H. L.
1912. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, 77 p., 9 fig., 2 pl.

(2) —— ——
1913. The water requirement of plants. I.—Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, 49 p., 11 pl.

(3) HELLRIEGEL, Hermann.
1883. Beiträge zu den naturwissenschaftlichen Grundlagen des Ackerbaus . . . Abschnitt 2.—Wurzel und Bodenvolumen. p. 118–280, 7 fig.

(4) KIESSELBACH, T. A.
1910. Transpiration experiments with the corn plant. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt. [1909], p. 125–139, 2 fig.

(5) KING, F. H.
1893. Natural distribution of roots in field soils. *In* Wis. Agr. Exp. Sta. 9th Ann. Rpt. [1891]/ 92, p. 112–120, fig. 9–17.

(6) ROTMISTROV, V. G.
1909. Root-Systems of Cultivated Plants of One Year's Growth. 57 p., illus. Odessa.

(7) SCHULZE, B.
1914? Studien über die Bewurzelung unserer Kulturpflanzen. *In* Festschrift, 50. Jubiläum Agr. Chem. Versuchs. u. Kontroll- Stat., Breslau, p. 67–95, 10 pl.

(8) SEELHORST, C. VON, and FRECKMANN, W.
1903. Der Einfluss der Wassergehaltes des Bodens auf die Ernten und die Ausbildung verschiedener Getreide-Varietäten. *In* Jour. Landw., Bd. 51, Heft 3, p. 253–269.

(9) TEN EYCK, A. M.
1899. A study of the root systems of wheat, oats, flax, corn, potatoes and sugar beets and the soil in which they grew. N. Dak. Agr. Exp. Sta. Bul. 36, p. 333–362.

(10) ———
1900. A study of the root systems of cultivated plants grown as farm crops. N. Dak. Agr. Exp. Sta. Bul. 43, p. 535–550, 15 fig.

(11) ———
1904. The roots of plants. Kan. Agr. Exp. Sta. Bul. 127, p. 199–252, 1 fig., 26 pl.

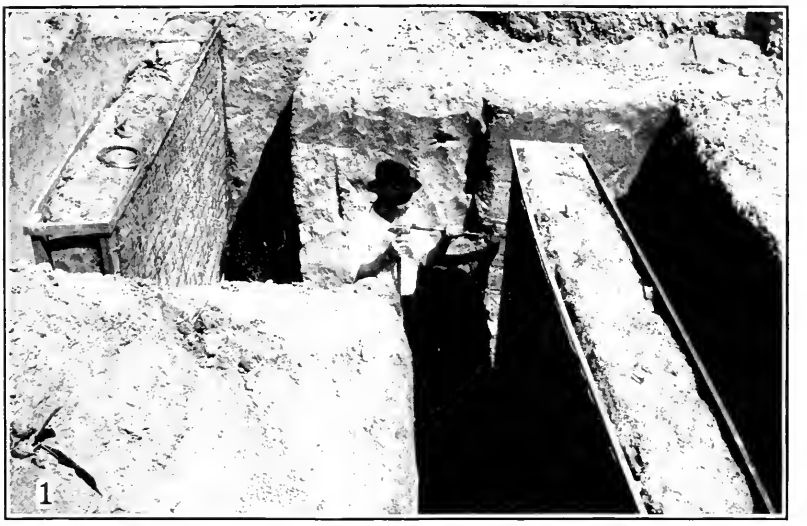
(12) TUCKER, M., and SEELHORST, C. VON.
1898. Der Einfluss, welchen der Wassergehalt und der Reichtum des Bodens auf die Ausbildung der Wurzeln und der oberirdischen Organe der Haferpflanze ausüben. *In* Jour. Landw., Bd. 46, Heft 1, p. 52–63.

PLATE XXXVIII

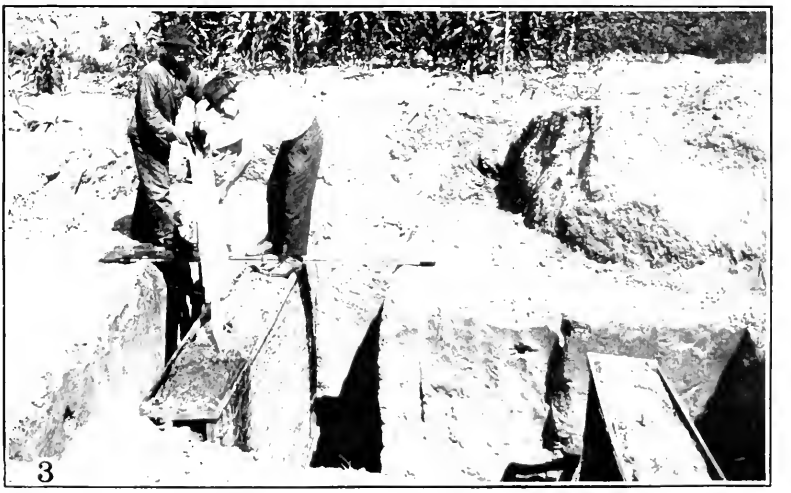
Fig. 1.—Method used in isolating root systems in the field. View of two soil prisms ready for washing. The trenches here shown are 3 feet wide, 12 feet long, and 6 feet deep.

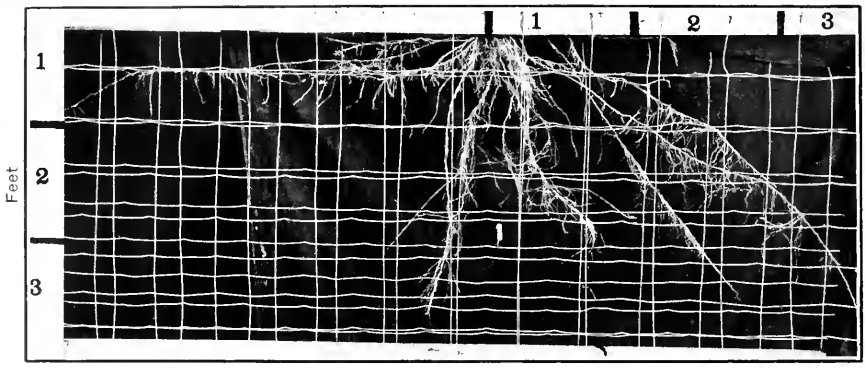
Fig. 2.—Method used in isolating root systems. This figure shows the method of placing the cross wires through the soil block.

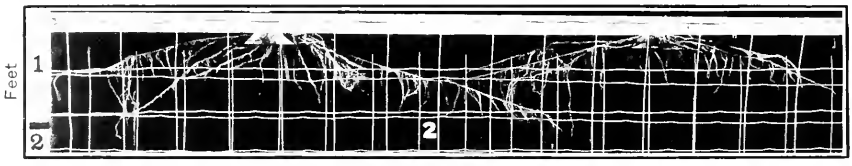
Fig. 3.—Method of washing used in the isolation of the root systems. The trench was partially filled with water, which was continuously pumped upon the prism of soil by means of a pitcher pump.

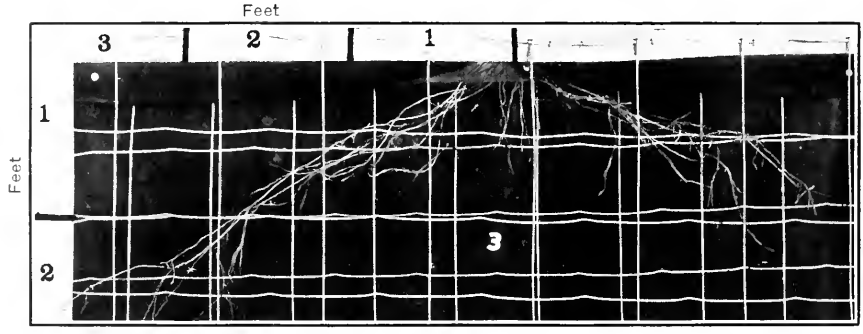

1


2


3


1
2
3
Feet
1
2
3
1


Feet
1
2
2


Feet
3
2
1
Feet
1
2
3

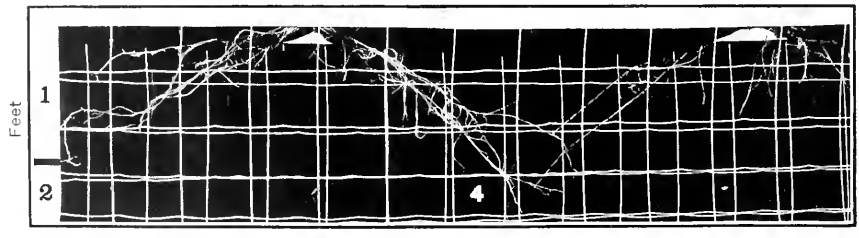

Feet
1
2
4

PLATE XXXIX

Fig. 1.—Root system of a corn plant that had reached a height of 3 feet 6 inches. Seed planted May 23, 1914. Root system isolated on July 17, 1914. Greatest depth of root penetration, 3 feet. Greatest lateral extent of the roots, 3 feet 6 inches.

Fig. 2.—Root systems of two corn plants with a height of 1 foot 6 inches. Seed planted on May 26, 1915. Root systems obtained on July 10, 1915. Greatest depth of roots, 1 foot 3 inches. Greatest lateral extent of roots, 2 feet 10 inches.

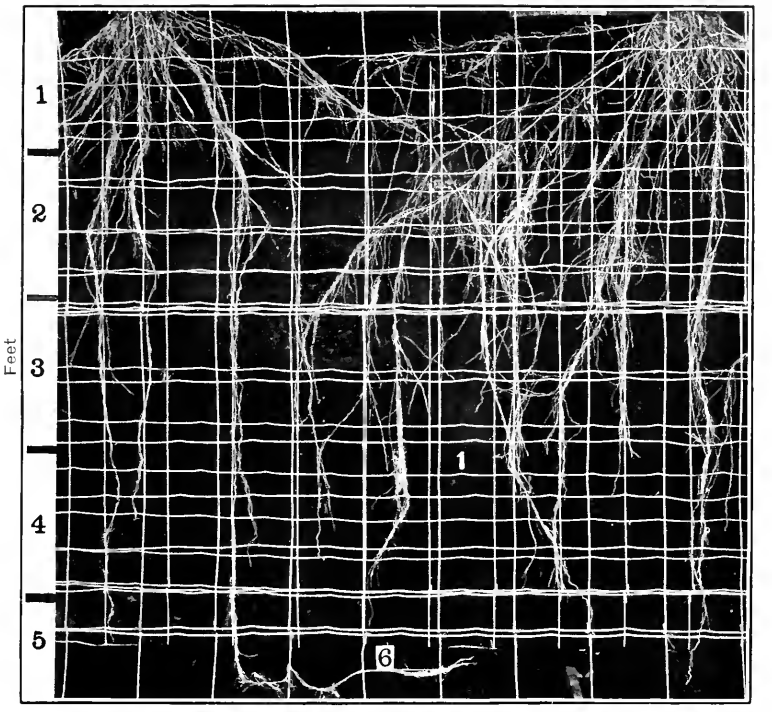
Fig. 3.—Root system of a Dwarf milo plant at the age of 4 weeks. Seed planted on May 23, 1914. Root system obtained on June 24, 1914. Plant stood 1 foot high. Greatest depth of root penetration, 1 foot 6 inches. Greatest lateral extent of roots, 3 feet.

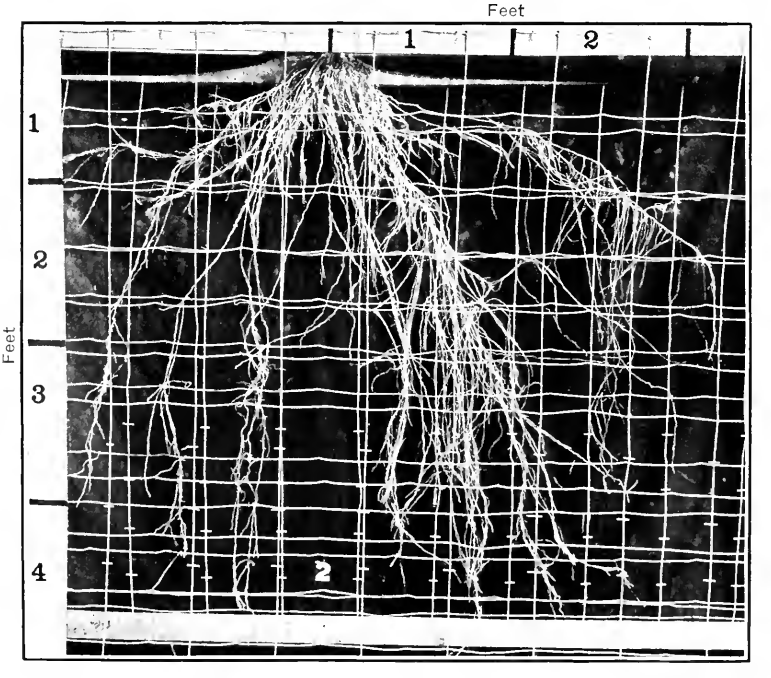
Fig. 4.—Root systems of two Blackhull kafir plants 1 foot in height. Seed planted on May 26, 1915. Root systems isolated on July 10, 1915. Greatest depth of root penetration, 1 foot 6 inches. Greatest lateral extent of roots, 2 feet.

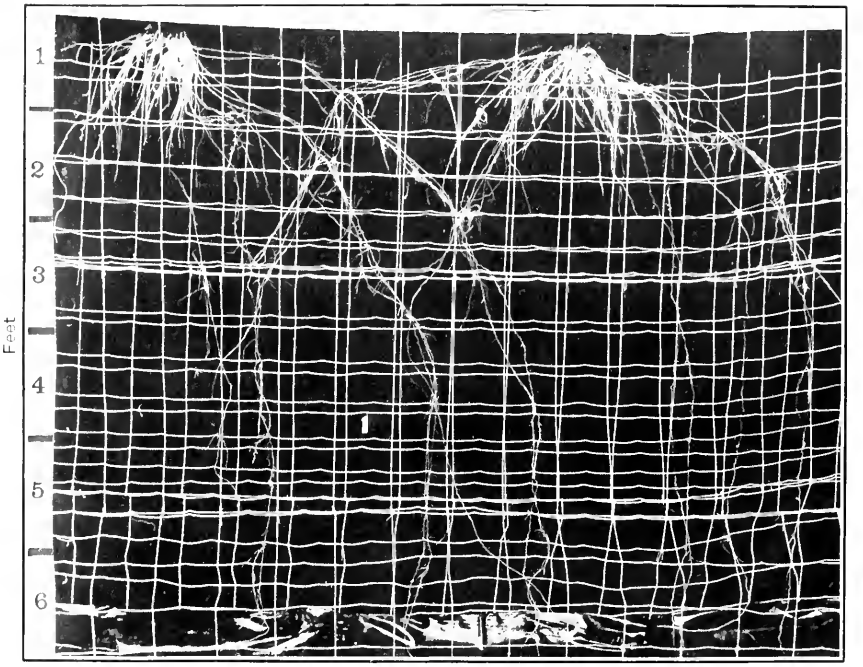
PLATE XL

Fig. 1.—Root systems of two mature corn plants. These plants stood 6 feet high, and the grain was in the glazed condition. Seed planted on May 23, 1914. Root systems obtained on August 25, 1914. Greatest lateral extent of the roots, 3 feet. Greatest depth of root penetration, 6 feet. The lower portion of the root cage is not shown here, but the roots which penetrated the sixth foot are shown in a horizontal position at the bottom of the cage.

Fig. 2.—Root system of a corn plant at the time of "shooting." Height of plant, 5 feet 6 inches. Seed planted on May 23, 1914. Root system obtained on August 1, 1915. Greatest depth of root penetration, 4 feet. Greatest lateral extent of the roots, 2 feet 6 inches.


Feet
1
2
3
4
5
1
6


Feet
1
2
Feet
1
2
3
4
2


Feet
1
2
3
4
5
6
1

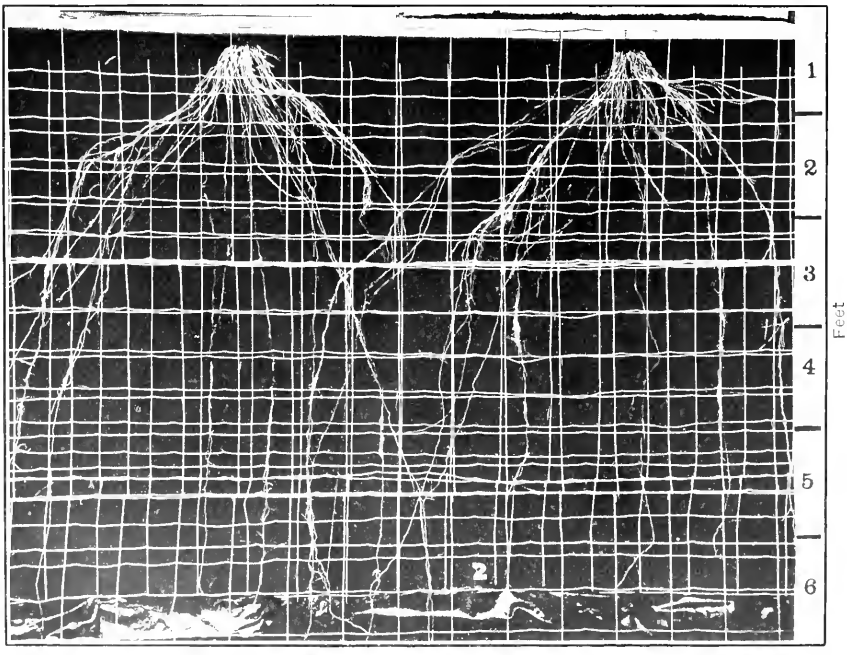

1
2
3
4
5
6
Feet
2

PLATE XLI

Fig. 1.—Root systems of two Blackhull kafir plants at the time they had reached a height of 6 feet and were blooming. Seed planted on May 26, 1915. Root systems isolated on September 3, 1915. Greatest depth of root penetration, 6 feet. Greatest lateral extent of the roots, 3 feet 8 inches.

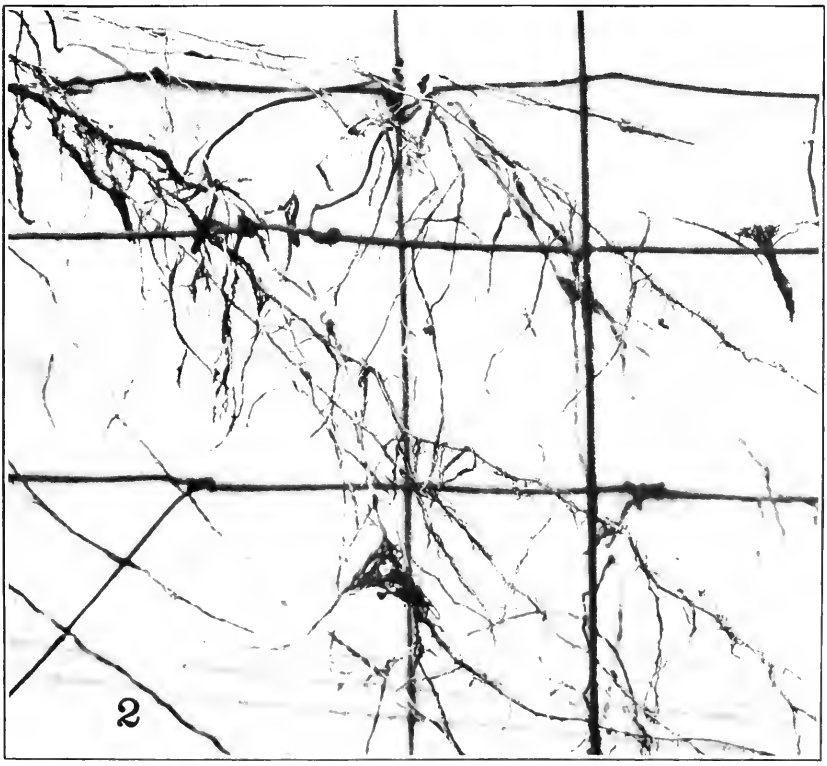
Fig. 2.—Root system of two Dwarf milo plants at the time the seed was in the milk stage. The plants stood 3 feet 6 inches high. Seed planted on May 26, 1915. Root systems isolated on September 3, 1915. Greatest vertical penetration of the roots, 6 feet. Greatest lateral extent of the roots, 3 feet 8 inches.

PLATE XLII

Fig. 1.—Portion of a primary root of Pride of Saline corn, showing the number and relative size of the secondary roots. Both the primary and secondary roots of the corn are larger than those of the Dwarf milo or standard kafir.

Fig. 2.—Portions of the primary roots of Blackhull kafir, showing the number and relative size of the secondary roots. Both the primary and secondary roots of Dwarf milo and Blackhull kafir are smaller and more fibrous than those of the corn. The number of secondary roots per unit of length of primary root is twice as great for Blackhull kafir and Dwarf milo as for the corn.


1


2


1

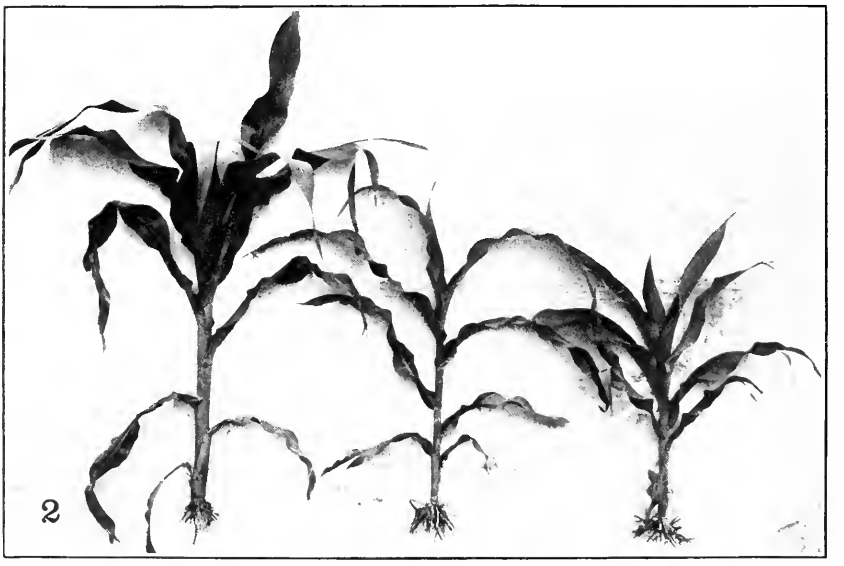

2

PLATE XLIII

Fig. 1.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 4 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on June 24, 1914.

Fig. 2.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 6 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on July 7, 1914.

PLATE XLIV

Fig. 1.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 8 weeks of age. Seed planted on May 23, 1914. Leaf areas determined on July 21, 1914.

Fig. 2.—Pride of Saline corn, Dwarf milo, and Blackhull kafir plants, showing their relative leaf and sheath areas at 10 weeks of age. At this time the plants have completed their leaf development. Seed planted on May 23, 1914. Leaf areas determined on August 4, 1914.


1


2

PRODUCTION OF CLEAR AND STERILIZED ANTI-HOG-CHOLERA SERUM

[PRELIMINARY PAPER]

By M. DORSET, *Chief*, and R. R. HENLEY, *Chemist, Biochemic Division, Bureau of Animal Industry*

INTRODUCTION

In the United States the anti-hog-cholera serum of commerce for the most part consists of the defibrinated blood of hyperimmunized hogs. The red corpuscles contained in such commercial serum are not only devoid of protective qualities but are objectionable for a number of reasons. The practice of using the defibrinated hog's blood was adopted because of the difficulty experienced in separating completely the clear serum from the fibrin and the blood corpuscles.

Hog blood, when allowed to undergo spontaneous coagulation, ordinarily yields but a small proportion of clear serum. In practice not more than 30 or 35 per cent can be secured, the remainder of the serum being held firmly within the large clot. If, instead of allowing the blood to clot spontaneously, immediate defibrination be practiced, a yield of defibrinated blood varying from 90 to 95 per cent may usually be obtained. This defibrinated blood contains all of the antibodies present in the blood when drawn, whereas, if the blood is allowed to coagulate and the separated clear serum alone is used, there must be a large loss of antibodies, because part of the serum is held back in the clot.

The occurrence of the foot-and-mouth disease in the United States and the accidental infection of certain lots of hog-cholera serum and virus with this disease have demonstrated the urgent need for some method of treating these products which will serve to remove the possibility of either of them being a medium for its dissemination. In order to insure the freedom of hog-cholera serum from the virus of the foot-and-mouth disease, it is not sufficient merely to filter the product through bacteria-proof filters, because the virus of this disease itself is known to pass through bacteria-proof filters. It is likewise known that the virus of the foot-and-mouth disease is more or less resistant to the preservatives which are commonly used and which are suitable for the preservation of serum. There seems to be, therefore, only one means by which the serum may be sterilized in so far as the virus of the foot-and-mouth disease is concerned, and that is by the application of heat. The best European authorities state that this virus is killed when heated at a temperature of 50° C. for 12 hours. It also seems

Journal of Agricultural Research, Vol. VI, No. 9
Dept. of Agriculture, Washington, D. C. May 29, 1916
em A—22

well established that the virus is killed by 5 minutes' exposure to a temperature of 60°.

Experimental work has shown that defibrinated hog-cholera-immune blood may be heated to 50° C. for 12 hours without destroying the antibodies and without materially altering the physical character of the defibrinated blood. Heating to higher temperatures—60°, for example—results in more or less complete coagulation of the defibrinated blood, and therefore in the destruction of the serum in so far as its commercial worth is concerned. While heating at 50° for 12 hours might appear to be satisfactory, in practice it would be difficult and expensive to carry out such a process.

Experiments with clear serum, separated from the red cells, have shown that, unlike the defibrinated blood, which coagulates at 60°, the serum, separated from the red blood cells, withstands heating at 60° for 30 minutes without alteration of its physical characters and without noticeable impairment of its antitoxic power.

With the above facts in mind, renewed efforts have been made to devise a cheap and simple process for preparing hog-cholera antitoxin in the form of a clear serum free from the red blood corpuscles and from corpuscular débris.

PREPARATION OF THE SERUM

If ordinary defibrinated hog's blood be subjected to centrifugalization, there may be secured ordinarily about 50 per cent of serum. The time required will naturally depend to a large extent upon the precipitating force developed by the centrifuge. We have found that a force equivalent to approximately 1,700 times gravity serves to attain this result in from 20 to 30 minutes. The serum which separates is usually cloudy, and, owing to the fact that the red blood corpuscles are not firmly packed, it is impossible to remove all of the serum without at the same time carrying over some of the red cells. Therefore, simple centrifugalization has not seemed practicable for the following reasons: (1) Antibodies are lost because of inability to separate all of the serum from the corpuscles, (2) the serum secured is generally not clear, and (3) the removal of the serum from the cells is a difficult and tedious procedure.

In endeavoring to overcome the difficulties enumerated above, we have used extracts of the seed of different varieties of the common garden bean (*Phascolus multiflorus* and *P. vulgaris*). Extracts of these beans are known to possess the property of agglutinating the red corpuscles of hog's blood, and they are said to be nontoxic.[1] Our own experience has shown that, although the extracts[2] exert no general systemic effect upon rabbits, guinea pigs, or hogs, certain varieties of these beans do yield extracts which act as intense local irritants, resulting, in guinea pigs

[1] Mendel, L. B. Observations on vegetable hæmagglutinins. *In* Arch. Fisiol., v. 7, p. 168–177. 1909.
[2] Extracts made with water or normal salt solution.

at least, in swelling, followed by necrosis of tissue and the formation of suppurating abscesses at the sites of injection. The extracts of the scarlet runner bean (*P. multiflorus*) and of the pink kidney bean (*P. vulgaris*) are both intensely irritating, while extracts of the common white navy bean (*P. vulgaris*) are entirely lacking in this irritating property. While both the scarlet runner and the kidney bean are very powerful agglutinants, they have been rejected, at least temporarily, and extracts of the common white navy bean have been used exclusively in our later work.

Very minute amounts of the extracts of the navy bean serve to agglutinate large quantities of defibrinated hog's blood; and when such agglutinated blood is centrifugalized, the red cells pack together and form a rather stiff jelly-like mass in the tube. With a precipitating force of about 1,700 times gravity about 50 per cent of serum may be separated in 15 minutes. The serum is clear and may be readily poured from the tube.

In order to secure a greater yield of serum and a more firmly packed clot of red corpuscles, we find that the addition of a small quantity of sodium chlorid is very effective. The addition of 1 per cent of sodium chlorid to defibrinated hog's blood after agglutination from the addition of bean extract has begun will increase the yield of serum from 50 per cent without the salt to 70 per cent when the salt is added.

Considerable experimental work has led to the adoption of certain conditions of work as being most favorable to the production of the maximum amount of clear serum from defibrinated hog's blood. While experience may later show that some changes in procedure are desirable, it seems best to describe here the exact method, which is now being applied in these laboratories, of producing a clear sterile serum, heated to avoid the possibility of foot-and-mouth disease infection.

PREPARATION OF BEAN EXTRACT.—One hundred gm. of coarsely ground white navy beans are allowed to soak for one hour in 500 c. c. of distilled water, with occasional stirring. The pulp is strained through cheesecloth or cotton and mixed with powdered kieselguhr and filtered until clear. A filter of paper pulp mixed with some kieselguhr has been found to be efficient. The clear filtered extract is passed through a bacteria-proof filter of infusorial earth.

PREPARATION OF DEFIBRINATED BLOOD FOR CENTRIFUGALIZING.—To each 100 c. c. of the cool defibrinated blood add 1 c. c. of the sterile bean extract and stir to secure a uniform mixture. Allow the mixture to stand until agglutination is clearly evident. This can be determined by examining a small amount in a glass or tube. Agglutination is usually apparent within five minutes after adding the bean extract. There should then be added 1 gm. of finely powdered sodium chlorid. The salt is stirred in until dissolved, and the mixture of defibrinated blood, bean extract, and salt is allowed to stand for about 15 minutes.

CENTRIFUGALIZING.—The defibrinated blood mixture is placed in suitable containers, preferably somewhat elongated, and rotated in a centrifuge for 15 minutes at a speed sufficient to produce in the cups a precipitating force equal to approximately 1,700 times gravity. At the end of this period the serum may be poured from the cups into suitable containers.

HEATING THE SERUM.—The clear serum obtained by centrifugalizing is placed in a container which is surrounded by a jacket of water. The temperature of the water in the outer jacket at the beginning of the heating should not exceed 63° C. The serum in the inner container is slowly stirred during the heating process, the temperature of the outer jacket being maintained between 61° and 62°. A thermometer should be kept constantly in the serum and care should be taken to see that the temperature of the serum, once it has reached 60° C., does not fall below that point and that it does not rise materially above it.[1] Continuous heating for 30 minutes at 60° C. is required. Upon the completion of the heating, the serum should be rapidly cooled. After cooling, 1 part of a 5 per cent solution of phenol should be added to 9 parts of the serum.

FILTERING THE SERUM.—After the phenol has been added a slight precipitate may at times form in the serum; therefore it is desirable to allow several days to elapse between the addition of the phenol and the final filtration through infusorial earth.

EXPERIMENTAL RESULTS

To illustrate the yield of clear serum obtained by the application of the described method to the preparation of anti-hog-cholera serum, there is given in Table I a statement of the yield of clear serum obtained from three different lots of defibrinated immune blood and one lot of defibrinated hog-cholera virus.

TABLE I.—*Yield of clear serum from defibrinated anti-hog-cholera serum and virus under a precipitating force of 1,700 times gravity applied for 12 minutes*

Blood.	Bean extract added.	Sodium chlorid added.	Serum yield.
	Per cent.	*Per cent.*	*Per cent.*
Hog-cholera serum from defibrinated immune blood 3895	None.	None.	47½
Do	1	None.	49
Do	1	1	70
Do	1	1	70
Serum from defibrinated immune blood 3866 and 2165	1	1	74
Serum from defibrinated immune blood 2166	1	1	70
Serum from defibrinated hog-cholera virus 377 and 379	1	1	78

Table II gives the results of potency tests of one lot of serum prepared by use of the bean and sodium chlorid mixture. As will be seen, a test was made of the whole defibrinated blood, of the clear serum separated

[1] Thermometers used should be standardized, and the temperature of the serum should not be allowed to exceed 60.5° C.

from such defibrinated blood by the use of bean extract and sodium chlorid, and of the cell residues from which the clear serum was removed. In preparing the cells for injection they were taken up in distilled water and made to a volume corresponding to the volume of defibrinated blood from which they were derived. Thus hog 2149 received all of the cell residue from 200 c. c. of defibrinated blood and hog 2150 received all of the cell material from 100 c. c. of defibrinated blood. The serum which was obtained from the defibrinated blood was used to inoculate hogs 2155 to 2158, inclusive.

TABLE II.—*Test of serum separated by use of bean extract and sodium chlorid in 1916*[a]

Hog No.	Weight.	Date inoculated.	Protective material injected.	Quantity of protective material injected.	Quantity of virus.	Results.	Date died.
	Pounds.			*C. c.*	*C. c.*		
2143	70	Mar. 24	Phenolized defibrinated blood 3895.	20	2	Remained normal throughout test.	
2144	65	...do....	Phenolized defibrinated blood 3895 (unwashed).	10	2	.....do.....	
2149	70	...do....	Cells from defibrinated blood 3895.	200	2	Injured in fighting Mar. 27; off feed Mar. 28 to Apr. 4. Very slight hemorrhagic lesions.	Apr. 4
2150	65	...do....	.....do.....	100	2	Went off feed Mar. 27; very sick Mar. 3 to Apr. 11. Extensive hemorrhagic lesions.	Apr. 11
2155	60	...do....	Clear serum from defibrinated blood 3895, heated.	16	2	Remained normal throughout test.	
2156	65	...do....	.....do.....	16	2	.....do.....	
2157	50	...do....	.....do.....	8	2	.....do.....	
2158	55	...do....	.....do.....	8	2	.....do.....	
2163	45	...do....	Control.....	.....	2	Well-marked lesions of hog cholera on post-mortem examination.	Apr. 12
2164	50	...do....	.....do.....	.....	2	Extensive lesions of hog cholera on post-mortem examination.	Apr. 11

[a] No inflammation or swelling at point of injection on any pigs in this test. Thriftiness of pigs remaining normal not impaired.

From the fact that both of the pigs injected with the cell material contracted hog cholera and died it seems clear that, in this experiment at least, the amount of antibodies left behind with the cells was negligible.

The bean-extract-sodium-chlorid method of separating the corpuscles from defibrinated hogs' blood has been applied repeatedly in these laboratories and always with success. There seems to be no reason why the process should not be entirely satisfactory for use in the practical production of anti-hog-cholera serum. There appears to be little or no loss in antibodies; the serum secured is generally clear; and it may be removed from the agglutinated cells easily by pouring from the cups. The method also would seem to tend toward a certain concentration of

the antibodies of the blood, and it is also to be recommended on account of the fact that it results in a large yield of serum.

The fact that this serum may be heated for half an hour at 60° C. without noticeable impairment of its potency is of much practical importance because there is thus afforded a ready means for safeguarding it against infection with the virus of the foot-and-mouth disease.

Anyone contemplating the practical application of the process is urged, at the beginning at least, to follow the method described herein, and to use only the common white navy bean for preparing the bean extract. It is hoped that the method will soon be adopted on a large scale by commercial producers of serum.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.

AT

15 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

Vol. VI JUNE 5, 1916 No. 10

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., JUNE 5, 1916 NO. 10

SILVER-SCURF OF THE IRISH POTATO CAUSED BY SPONDYLOCLADIUM ATROVIRENS

By EUGENE S. SCHULTZ,[1]
Expert in Potato Investigations, Cotton and Truck Disease Investigations, Bureau of Plant Industry

INTRODUCTION

Silver-scurf of the Irish potato (*Solanum tuberosum*), caused by *Spondylocladium atrovirens*, has been known in Europe since 1871, when it was discovered by Harz (6) on new potatoes in Vienna; but there is no record of its appearance in this country until mentioned by Clinton (4) in 1908. Notwithstanding its comparatively recent discovery, its general distribution in the eastern United States was shown by Melhus (7), 1913, who also raised the question as to its importance as a new potato disease in America, while its appearance in the Northwest was first reported in 1914 by Bailey (2) and later, in 1915, by O'Gara (8).

Reports of studies made by former investigators contain contradictory assertions, especially on the effect of this organism upon the host. It is evident, therefore, that further study of the symptoms, manner of infection, and physiology of the organism is desirable in order to understand more fully the significance of this disease, which has already become widely distributed in this country.

STUDIES OF THE FUNGUS

MORPHOLOGY

Spondylocladium atrovirens, one of the black molds, is classified according to Saccardo (9, p. 483) in the Fungi Imperfecti under the Dematieae. The genus Spondylocladium is characterized by its dark multiseptate conidiophores, which bear the many-celled conidia pleurogenously in the form of whorls.

Conidiophore and conidia formation can be studied either in hanging-drop or agar cultures. When the organism was cultured on agar plates

[1] The sincere thanks of the author are due to Dr. I. E. Melhus, Bureau of Plant Industry, for many helpful suggestions during the progress of this study and the preparation of the manuscript, and also to Prof. L. R. Jones, of the University of Wisconsin, in whose laboratory a part of the work was conducted.

held at room temperature, conidiophores and conidia appeared in 10 to 12 days, which indicates that *S. atrovirens* is one of the slow-growing fungi.

The conidia are formed first either at the apex or the distal end of the intermediate cells. Under certain apparently abnormal conditions, however, they appear at the ends of what seem to be ordinary branches of the mycelium, but in that case the character of the normal conidiophore is absent. The lowest whorls of conidia are borne about halfway between the base and the apex of the conidiophores, and the conidia are attached at the broad end (Pl. XLVI, fig. 2).

Germination of the conidia takes place by means of germ tubes. These are produced from either pole, generally from the distal or pointed end, as well as from any cell of the conidium, as observed by previous investigators. Germination in water occurs within 24 to 40 hours; and in a few days the somewhat hyalin, knoblike protrusion, which is characteristic of the early stages of germ formation, develops a multiseptate, branched mycelium which is of a much lighter color than the conidiophores, conidia, or portions of the old mycelium. This is very hyalin and continues so up to the time of conidiophore formation, at which time dark-brown, thickened cells are formed in different parts, and from these specialized cells are produced the many-septate, dark-brown conidiophores, which attain a length of 5 mm. and are perceptibly wider than the surrounding mycelium (Pl. XLVI, fig. 1).

Because of the wide variation found in the size of the spores, Appel and Clinton (4, p. 359) suggested the possibility of there being two species of the fungus—that is, a large-spore and a small-spore species. Several series of 18 measurements were made by the writer on conidia taken from tubers imported from Germany and tubers from various parts of the United States. A wide variation in dimensions occurred in the conidia from all the various tubers used in the experiment. The conidia taken direct from the surface of the tuber from Germany varied from 22 to 42μ (mostly 30 to 40) in length, 6 to 12μ (mostly 6 to 8) in width at greatest diameter, and were 4 to 8 (mostly 5 to 6) septate; conidia taken from the progeny of tubers from Maine grown in Washington, D. C., varied from 30.4 to 56.2μ (mostly 30 to 40) in length, 7.6 to 9.5μ (mostly 7.6 to 8.5) in width, and were from 4 to 7 septate; while the conidia taken from tubers from Rhode Island, West Virginia, Washington, D. C., Oregon, Washington, and Wisconsin averaged 32.6 to 40μ in length, 7.5 to 8.5μ in width, and were 5- to 7-septate.

In order to study more fully the variation of spore dimensions, several series of measurements were made on conidia produced from a single spore strain. The difference in dimensions obtained in this case ranged from 18 to 64μ (mostly 30.4 to 40) in length, and 7 to 8.1μ in width, and 5 to 6 septa.

From this it is apparent that, even though considerable variation in spore dimensions occurred on infected tubers from different localities, nevertheless an even greater variation resulted in the case of spores from a single spore strain. This shows that normally a wide variation exists, and consequently it does not appear necessary to form small-spore and large-spore species.

REACTION OF THE FUNGUS TO LIGHT

In order to secure a better knowledge of the relation of *S. atrovirens* to its environment so that its life history might be better understood, experiments on some of the physiological characteristics of this organism were conducted.

The reaction to light is of special interest in connection with the effect of storage conditions upon the development of the fungus on potatoes.

In this study the writer used the plate-dilution method, the conidia being sufficiently diluted on Lima-bean agar plates to be observed individually. Immediately after the plates were poured, each was wrapped in carbon paper, the entire dish being covered except an aperture from 1 to 2 cm. in diameter at the side, and the plates were then arranged with the apertures facing the light from the window.

The plates were examined at the end of three days and it was found that the mycelial branches developed on the side of the hyphæ farthest away from the window and that the majority of these grew in the opposite direction from the source of the light. The position of germ-tube formation does not appear to be influenced by the light, germination sometimes taking place from the side closest to the source of light; but as soon as the germ tube receives the heliotropic stimulus—that is, when it is a few millimeters long—it invariably turns away from the light, and subsequent mycelial development is formed on the side of the conidium farthest from the source of light. Instead of appearing at the center of the colony, therefore, the conidia are found at the margin exposed to the light, and at the end of 5 to 10 days the entire colony appears as if a gentle breeze had blown the hyphæ in one general direction away from the light (Pl. XLVI, fig. 3). These results also confirm Eichinger's (5) observations.

The reaction of this fungus to light in culture media demonstrated that it is negatively heliotropic. In view of the fact that infection of the tubers in the field takes place in the dark, negative heliotropism here does not obtain. In order to determine whether this heliotropic property favored tuber infection, artificial inoculations were made on tubers in the light. In this case no perceptible difference occurred, since infection appeared on all parts of the tubers alike.

REACTION OF THE FUNGUS TO MOISTURE

Like most fungi, *S. atrovirens* requires considerable moisture for development; but, owing to the absence of accurate instruments for

measuring the degree of moisture, only approximate data regarding moisture reaction can be given. It was noted in field studies that a higher percentage of infection occurred in the lower and more moist sections of the field than in the higher areas, and that in laboratory infection experiments the fungus develops best when the surface of the tuber is kept moist but not supersaturated. By placing tubers sufficiently near water so that a heavy film of moisture was constantly present, it was found that sporulation was inhibited to a greater degree on the side of the tuber near the water than on the opposite side, which indicates that excess moisture may check the growth of the fungus.

Although the fungus prefers moisture for growth, it can withstand drying without the entire loss of its virility. This was shown by the fact that transfers from agar cultures 16 months old continued to grow, although only a small percentage of the conidia germinated. Notwithstanding the fact that these cultures had been kept at room temperature and were dried to such an extent that simply a dry, brittle mass of media and fungus remained, both viable conidia and mycelium were found.

REACTION OF THE FUNGUS TO TEMPERATURE

Conidia in corn meal and oat agar and in water and naturally infected and artificially inoculated potato tubers were used in studies to determine the effect of temperature on *S. atrovirens*. In the case of media spore-dilution plates were prepared, the spores being sufficiently far apart so that individual colonies were retained. The same dilution was used on each plate and all were inoculated at temperatures ranging from 2° to 31° C. The water cultures were used in making hanging-drop preparations on Van Tiegham cells and in small Petri dishes, the spore suspensions in this case also being made in such manner that some of the spores remained on the surface, although germination occurred to a slight extent also beneath the surface. The naturally infected and artificially inoculated tubers were placed in pint bottles containing some pebbles and a few cubic centimeters of water, with a piece of cheesecloth extending from the contents of the bottle to its mouth, thus forming a moist chamber. These bottles were incubated in the same way as the media cultures.

In the eight series of Petri-dish cultures microscopic germination was noted at 3°, 4°, and 5° C., but no macroscopic colonies developed; at temperatures ranging from 6° to 28° macroscopic colonies were obtained, 21° to 27° being the optimum for abundance of growth; while at 30° or 31° no macroscopic growth was apparent (Pl. XLVII). These temperature limits for growth were confirmed by the water cultures, which were used as checks on the media cultures subjected to the highest and the lowest temperatures. In the case of three series of these water cultures which were subjected to a temperature of from −5° to −10° C. for four days and then brought to room temperature, 80 per cent of the

conidia germinated within 48 hours, and pieces of the mycelium in the cultures also showed growth. Agar culture and cultures on sweet-clover stems subjected to the same temperature also remained viable, as indicated by subsequent transfers, hanging-drop cultures showing that both conidia and mycelium retained their vitality.

In the test with naturally infected and artificially inoculated potatoes sporulation occurred on the former at temperatures ranging from 6° to 27° and on the latter at a range of from 12° to 27° C. In cultures on agar media and sweet-clover stems subjected to 35° and 50° further growth was inhibited at the former temperature, but the fungus remained alive after two weeks' exposure, while it was killed when subjected to 50° for three days.

REACTION OF THE FUNGUS TO MEDIA

Since *S. atrovirens* is a relatively slow-growing organism, tests were made with media of different grades of acidity with a view of facilitating growth in culture. The media used for this purpose were synthetic, Lima-bean, string-bean, oat, potato, corn-meal, and beef agar, all of which varied in reaction from +15 to −15 Fuller's scale.

Two plates each of these media equally diluted with conidia from the same culture were poured, and all were incubated at room temperature. Examinations of the colony development, including nature and extent of growth and sporulation, were made at 4-, 6-, and 12-day intervals and showed that *S. atrovirens* developed slightly faster on potato and Lima-bean agar than on string-bean, corn-meal, or oat agar; that growth was much retarded on beef agar; that mycelial development was very decidedly inhibited on synthetic agar; that sporulation occurred slightly sooner on oat agar than on the other agars; and that the hyphæ on fruiting remained lighter in color on Lima-bean and beef agars than on other agars.

The optimum reaction appeared to depend largely on the kind of medium. On potato agar no perceptible difference in growth appeared between +10 and −10, but mycelial development was much retarded at +15. On corn-meal agar only +1, 0, −1, −3, −5, and −15 reactions were run, because of the fact that hydrolysis took place when there was a higher degree of acidity. In this series +1 reaction was the optimum for growth, and in this case the mycelium became dark earlier than was the case in the minus reactions, owing possibly to the hydrolytic action of the acid on the media. On Lima-bean agar with +5 to −3 reactions the apparent growth of the fungus was not much changed, but with 5 to 10 and −3 to −10 reactions mycelial growth was perceptibly retarded. On beef agar optimum reactions ranged from 0 to +1, very little difference appeared in the colonies at +3 to −3, growth was gradually retarded at 5 to 15, and no colonies were macroscopically visible at the end of 10 days on reactions ranging from −5 to −15.

Besides this test of different reactions of the medium, a series of nutrition tests was conducted, a full nutrient agar, including carbon, nitrogen, oxygen, hydrogen, potassium, phosphorus, magnesium, sulphur, and iron, being used. With one exception each set of the media contained one element less than the full nutrient culture; in other words, the experiment was arranged as follows: (1) Check containing water agar, (2) full nutrient, (3) full nutrient minus nitrogen, (4) full nutrient minus potassium, (5) full nutrient minus phosphorus, (6) full nutrient minus magnesium, (7) full nutrient minus sulphur, (8) full nutrient minus iron, (9) full nutrient minus carbon, (10) full nutrient minus all minerals. Two plates of each kind of agar were inoculated with conidia and two with mycelium from the same culture of *S. atrovirens*, and all were incubated in the laboratory at room temperature.

Examinations at the end of 15 and 20 days indicated that sporulation occurred only on the plates from which sugar was omitted—that is, Nos. 1 and 9—the colonies on these plates being of a light color and spreading character and from 1.5 to 2.5 cm. in diameter and that no sporulation occurred on the plates from which sugar had been omitted, the mycelium in these being dark and densely compacted and only 0.75 to 1.25 cm. in diameter.

This preliminary study of the reactions of media on *S. atrovirens* indicates that neutral or slightly acid reactions are more favorable for the growth of this fungus; that the kind of medium determines the effect of higher reactions on this organism as shown by the alkaline reactions of beef agar compared with the same reactions of potato or the other agars; that compounds in one kind of medium may be formed which are seemingly toxic, whereas in a different kind of medium the same adjustment produces no such inhibitory effects; and that the presence of 5 per cent of cane sugar in a nutrient agar inhibited sporulation, but induced dark, heavy, compact mycelial growth, while the absence of sugar caused sporulation and a more spreading mycelial development.

HISTOLOGY

Studies were made to determine the relation of *S. atrovirens* to the potato. Both normal and affected material from the eye end of Irish Cobbler, Green Mountain, and Minnesota Triumph tubers badly infected normally and artificially was taken from the center and from the margin, that from the latter with and without lenticels or eyes. This material was embedded, sectioned, and stained according to ordinary cytological methods. From these studies it was evident that the mycelium may enter the tuber through the lenticels or between the lenticels through the epidermis.

After the fungus gains entrance the hyphæ invariably form within the cells, where they appear as a single branch of the mycelium; or they

may shorten and thicken to form a short and many-celled mass of hyphæ, from which the conidiophores subsequently arise. In severe cases of infection the cells appear to be disintegrated by the invasion to such an extent that only two or three instead of six or more cork layers remain above the living parenchyma. In experiments with potato roots grown under sterile conditions and inoculated with conidia and mycelium of the fungus, the mycelium grew on the surface, but did not penetrate the parenchyma, which indicates that the roots are less subject to infection than the tubers.

So far as the author has been able to determine, the fungus hyphæ confine their activity to the corky layers. In no case has it been found in the living parenchyma. This superficial infection causes a loosening of the corky and epidermal cell layers, so that these subsequently slough off. In this manner transpiration may proceed with greater facility and thus affect the parenchyma layers.

That *S. atrovirens* prefers this relatively heavy corky layer is further apparent from the fact that it grows very sparingly on the cut surface of the tubers where the loosened surface cells are invaded. Furthermore, its very limited presence on roots, stems, and stolons also indicates that it prefers the heavier, corky layers of the potato tuber.

EFFECTS OF THE FUNGUS ON THE HOST

The progress of the disease after tuber infection may be divided into two stages, the early and the late. In the former the infected areas are light-brown and have a glazed appearance, the latter characteristic becoming especially pronounced when the infected surface is moistened. Sometimes the margins of these areas are slightly fimbricated. The discoloration, which is found on newly infected tubers at harvest time, is often so inconspicuous as to pass unnoticed, even on close examination, unless the tubers are washed. When infected tubers are placed in moist chambers, the brownish areas become olive-colored, owing to the formation of conidiophores and conidia. The late stage is characterized by the shrinking and shriveling of the diseased areas and sloughing off of the epidermis and may be subdivided into two stages: The spot or patch infection (Pl. XLV, fig. 2) and general infection (Pl. XLV, fig. 1). In the former slightly sunken isolated areas on the surface show the shriveling, and late in the storage season these areas become shriveled and sunken.

In the case of general infection the entire surface is covered with infected areas and the epidermal and corky layers may shrink to such an extent that distinct folds or ridges appear. In the red-skinned varieties the color is completely destroyed. This again largely only mars the appearance and not their food value, but still they must be sold at a sacrifice. Potatoes stored under moisture and temperature

conditions favorable to sporulation often become so badly infected that they become a dull-black, the tubers having the appearance of having been dusted with soot. Several such bins were observed in Maine in May and June, 1914.

In case of slight infection in the field the infected areas are often found in isolated spots close to the stem end of the tuber. This was the case in practically every infected tuber harvested from the silver-scurf experimental plot at Caribou, Me., in the fall of 1914 and coincides with the observations of Appel and Laubert (1). While no reasons for this phenomenon are given by these investigators, from experiments and observations so far made it appears that infection is brought about through contact of the stem end of the young tuber with the infected mother tuber (Pl. XLVIII). This is indicated by the fact that in many cases where there was but slight contact only small areas about the point of the stolon attachment showed infection, while in the case of extensive contact infection was more widespread. It is further indicated by the fact that only one or two tubers closest to the mother tuber showed infection in counts made when the crop was about three-fourths grown, while in counts made later, after the conidia had become generally distributed, a large percentage of the tubers were infected.

Although infection appears to take place through the stem end, both stem ends and eye ends are subject to infection, general infection of both resulting from artificial inoculations.

In view of the fact that investigators like Bohutinsky (3) have attributed to *S. atrovirens* foliage symptoms such as leafroll, mosaic, etc., inoculations upon stems, stolons, and roots of the potato plant were made, both under field and greenhouse conditions. Two distinct procedures were followed: In one set of experiments viable spores were sprayed upon the stems, stolons, and roots; in the other virile mycelium was inserted into the inoculated portions. Checks were also run. Experiments in this order were run during 1914 and 1915, and in every case the inoculated plants behaved like the checks—viz, no perceptible infection occurred—showing again the inability of this organism to invade the vine tissues of the host.

METHODS OF DISSEMINATION

The fungus lives over by means of the mycelium, conidia, and sclerotia within the infected areas, so that under favorable conditions of moisture and temperature sporulation occurs and infection may spread even in storage. Not only do the infected tubers carry the disease to new sections, but they may carry it over from one season to another in the soil and in this way infect the new crop. This was the case in the author's field studies in Maine, viable conidia being found on the surface of

mother tubers taken on August 2, 1914, the date of the last examination, from an oat field at Houlton, in which they undoubtedly over-wintered in the soil. Many of these volunteer plants occurred in fields in which rotation had not been practiced, the deep snows which covered the ground the previous winter having protected the tubers.

Whether the fungus may live over in the soil from which the tuber host has been removed is not yet known, but that it may do so is not improbable, in view of what occurs in the case of fungi having a similar life history. Investigations to determine this point are now in progress.

Several series of experiments were undertaken to ascertain how readily *S. atrovirens* spreads from infected to healthy tubers and whether infection in this way might occur during the entire storage season. Inverted bell jars were used in these experiments to secure moist chambers which would hold a sufficient number of tubers for a satisfactory test and at the same time retain uniform moisture conditions. A wire rack of ¼-inch mesh was placed in each jar to support the potatoes and to prevent contact with the water in the jars, the inside of each jar was lined with blotting paper to conserve the moisture and prevent the entrance of excessive light, and the mouth was covered with window glass. Four varieties of potatoes were used: Rural New Yorker, Green Mountain, Irish Cobbler, and Bliss Triumph. A spore suspension of conidia which had been grown in pure culture on sweet-clover stems for four weeks was sprayed on the tubers with an atomizer, and for several days thereafter water was sprayed into the jars with the atomizer to keep the air saturated. A similar lot of healthy tubers was arranged as a check.

The first series was begun at Houlton, Me., on March 26, 1914; and within three weeks the entire surface of the inoculated tubers was covered with dark-brown conidiophores and conidia, while the checks were free from infection. Additional tests were made at Caribou, Me., on July 20, 1914; Washington, D. C., in December, 1914; Madison, Wis., on March 25, 1915; and Presque Isle, Me., on August 2, 1915. In each case infection occurred within three weeks after inoculation.

Similar infection experiments were conducted upon young tubers just harvested, as well as upon tubers still attached to the vines. In case of the tubers attached to the vines the soil was removed and a spore suspension was applied with an atomizer, whereupon the tubers were again covered with earth. Checks also were made. In each of these tests infection appeared upon tubers varying in diameter from 1 and 2 cm. to full-grown tubers (Pl. XLVII). Checks showed no infection.

From these results it is apparent that infection from *S. atrovirens* may take place at any stage in the development of the tubers and at any time throughout the storage season.

METHODS OF CONTROL

Melhus (7) found in laboratory experiments that neither double strength of mercuric chlorid (1:500) nor formalin applied for longer than the ordinary periods would completely inhibit the development of *S. atrovirens* on the potato and that both injured the tubers to such an extent that germination was decidedly inhibited. He also found that in many cases sporulation was inhibited on the surface of infected tubers treated with solutions of mercuric chlorid heated by a method devised by him for heating the solution for brief periods at temperatures near the thermal death point of protoplasm.

In view of these results, field tests were conducted during 1914 and 1915, both in Maine and at Norfolk, Va. Infected tubers were treated in double strength and heated solutions of mercuric chlorid. In Maine the treated tubers were planted on virgin soil.

As noted in Table I, the temperature fluctuated slightly, owing to the lower temperature of the tubers than that of the solution in which they were immersed. This table indicates that there was a decrease in the percentage of infected progeny in the treated rows as compared with the check. However, in no case was there a complete control of the infection. Similar tests in 1915 also indicated that even though silver-scurf may be inhibited to some extent; nevertheless, no treatment served as a complete control.

TABLE I.—*Effect of warm solution of mercuric chlorid on silver-scurf of the Irish potato*

Row No.	Strength of solution.	Temperature of solution.	Time of immersion.	Number of hills.	Number of hills infected.	Per centage of hills infected.	Average percentage infected hills in rows 14, 15, and 16.	Weight of healthy tubers.	Weight of infected tubers.	Percentage of infected tubers.	Average percentage of infected tubers in rows 14, 15, and 16.
	Per ct.	°C.	*Min.*					*Pounds.*	*Pounds.*		
13...	.....	Control.	.....	78	40	51. 28	.....	85	11. 25	11. 67	.....
14...	0. 2	47–52	5	63	29	46. 03	.....	84	10	10. 13	.....
15...	. 2	47–48	5	93	6	6. 45	21. 04	102	1	. 98	4. 91
16...	. 2	45–49	10	75	8	10. 66	.....	75	2. 5	3. 21	.....

In October, 1914, four pecks of tubers infected with S. *atrovirens* were subjected to a 1 to 1,000 solution of mercuric chlorid ranging from 45° to 53° C. for four minutes with a view of ascertaining the effect of treating infected tubers with that solution before storing. After treatment the tubers were placed in new muslin peck sacks and part of the lot stored at Caribou and part at Washington. At the same time separate lots of untreated infected and clean tubers were stored. On exami-

nation of these lots in June, 1915, the fungus was found fruiting on both treated and untreated infected tubers, but no infection was found on the untreated clean tubers.

As the treatments described do not absolutely control silver-scurf and as clean tubers only escaped infection, it is evident that disease-free seed should be selected in the fall and should be kept from contact with infected tubers in storage. Moreover, in view of the inhibitory effect of very low temperatures on the development of the fungus, the tubers should be stored at the lowest temperature permissible.

SUMMARY

A study of silver-scurf of the Irish potato, caused by *Spondylocladium atrovirens* Harz, shows that, notwithstanding the wide range in spore dimensions, which led certain investigators to believe there might be a large-spore and a small-spore species in this country, there is but one species, as proved by the fact that conidia ranging from 18 to 64μ were produced by a single spore culture.

S. atrovirens is negatively heliotropic. This, however, does not materially influence tuber infection in nature.

Severe drying of the conidia and mycelium in agar culture at room temperature does not kill the fungus.

S. atrovirens withstands a wide range of temperature. Its growth is inhibited at 2° to 3° C., but it is not killed at − 10°. Its optimum temperature is 21° to 27°, maximum 30° C.

Optimum reaction to media varies with the kind used, neutral to slightly acid reactions being most favorable to the development of the fungus. Five per cent of cane sugar in nutrient agar inhibited sporulation.

The fungus enters the tuber through the lenticels or the epidermal layers between the lenticels. The mycelium invades and disorganizes the epidermal and corky layers, leaving in bad cases only one or two instead of six or more layers, thus apparently accelerating transpiration.

The disease may be carried from place to place by infected tubers, in which it lives over from one season to another, or to the succeeding crop by the infected tubers which remain in the field over the winter.

Under favorable moisture and temperature conditions potatoes may become infected throughout the entire storage season. Both old and young tubers are subject to infection.

Inoculations on living stems, stolons, and roots in the field and laboratory experiments produced no infection.

Warm solutions of mercuric chlorid have a more toxic effect on *S. atrovirens* than cold solutions.

LITERATURE CITED

(1) APPEL, Otto, and LAUBERT, R.
1907. Die Konidienform und die pathologische Bedeutung des Kartoffelpilzes Phellomyces sclerotiophorus Frank. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 5, Heft 7, p. 435–441, pl. 11.

(2) BAILEY, F. D.
1914. Notes on potato diseases from the northwest. *In* Phytopathology, v. 4, no. 4, p. 321–322, pl. 10.

(3) BOHUTINSKY, G.
1910. Beitrage zur Erforschung der Blattrollkrankheit. *In* Ztschr. Landw. Versuchsw. Oesterr., Jahrg. 13, Heft 7, p. 607–633, 3 fig.

(4) CLINTON, G. P.
1908. Scurf, Spondylocladium atrovirens Harz. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 31/32, [1907]/08, p. 357–359. Quotes (p. 359) statement by Appel.

(5) EICHINGER, A.
1909. Zur Kenntnis einiger Schalenpilze der Kartoffel. *In* Ann. Mycol., v. 7, no. 4, p. 356–364, 3 fig.

(6) HARZ, C. O.
1871. Einige neue Hyphomyceten Berlin's und Wien's nebst Beiträgen zur Systematik derselben. *In* Bul. Soc. Imp. Nat. Moscou, t. 44, pt. 1, p. 129.

(7) MELHUS, I. E.
1913. Silver scurf, a disease of the potato. *In* U. S. Dept. Agr. Bur. Plant. Indus. Circ. 127, p. 15–24, 4 fig.

(8) O'GARA, P. J.
1915. Occurrence of silver scurf of potatoes in the Salt Lake Valley, Utah. *In* Science, n. s. v. 41, no. 1047, p. 131–132.

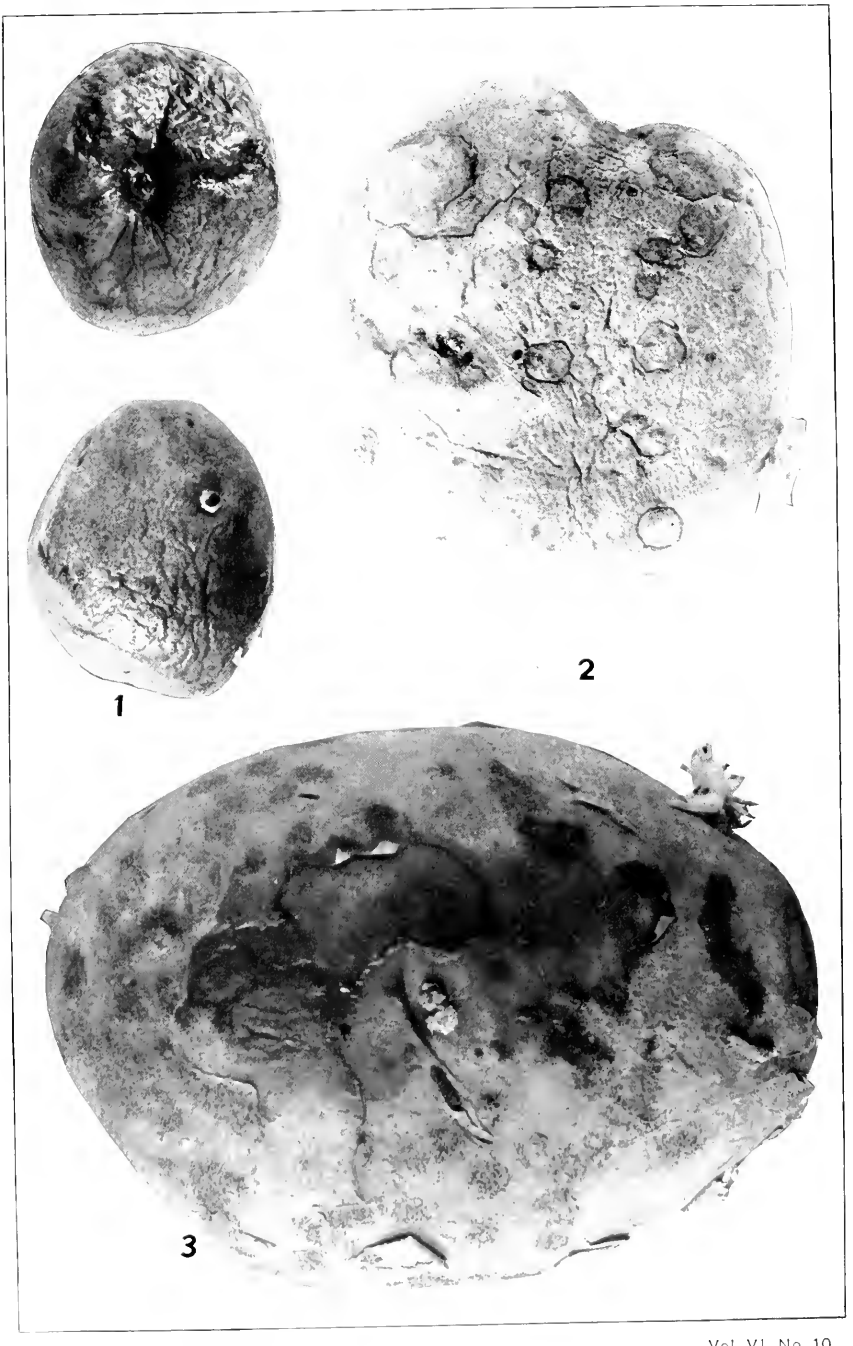
(9) SACCARDO, P. A.
1886. Sylloge Fungorum ... v. 4. Patavii.

PLATE XLV

Fig. 1.—Potato tubers showing shriveling and a silvery appearance caused by *Spondylocladium atrovireus*.

Fig. 2.—Tuber naturally infected by *S. atrovirens*, showing the segregated area type of infection, a condition developing in some cases later in the storage season.

Fig. 3.—Immature potato tuber artificially inoculated with conidia of *S. atrovirens*, July, 1913, at Houlton, Me. Infected area covered with dark-brown tufts of conidiophores and conidia. Infection was effected in a moist chamber at room temperature.


1
2
3

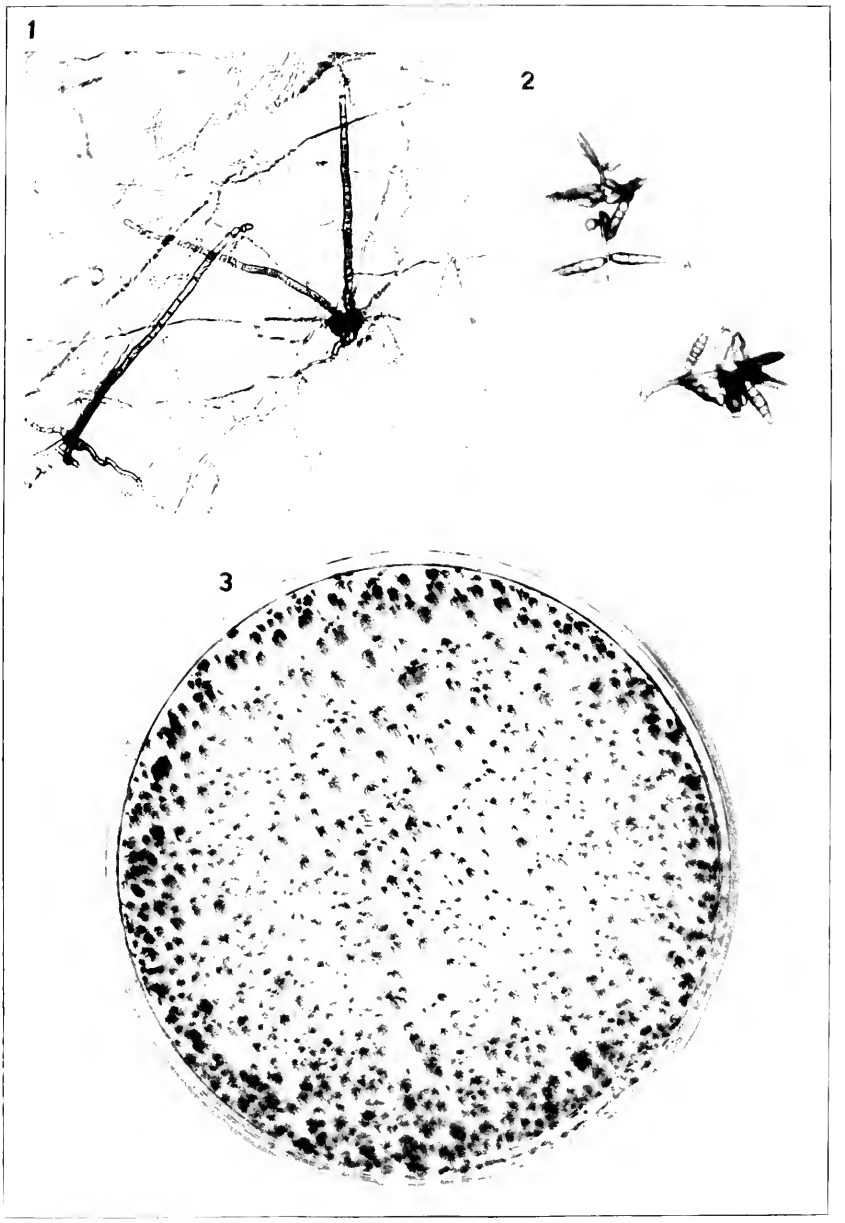

1
2
3

PLATE XLVI

Fig. 1.—Photomicrograph of *Spondylocladium atrovirens* on corn-meal agar, showing method of development of conidiophores and conidia in the early stages.

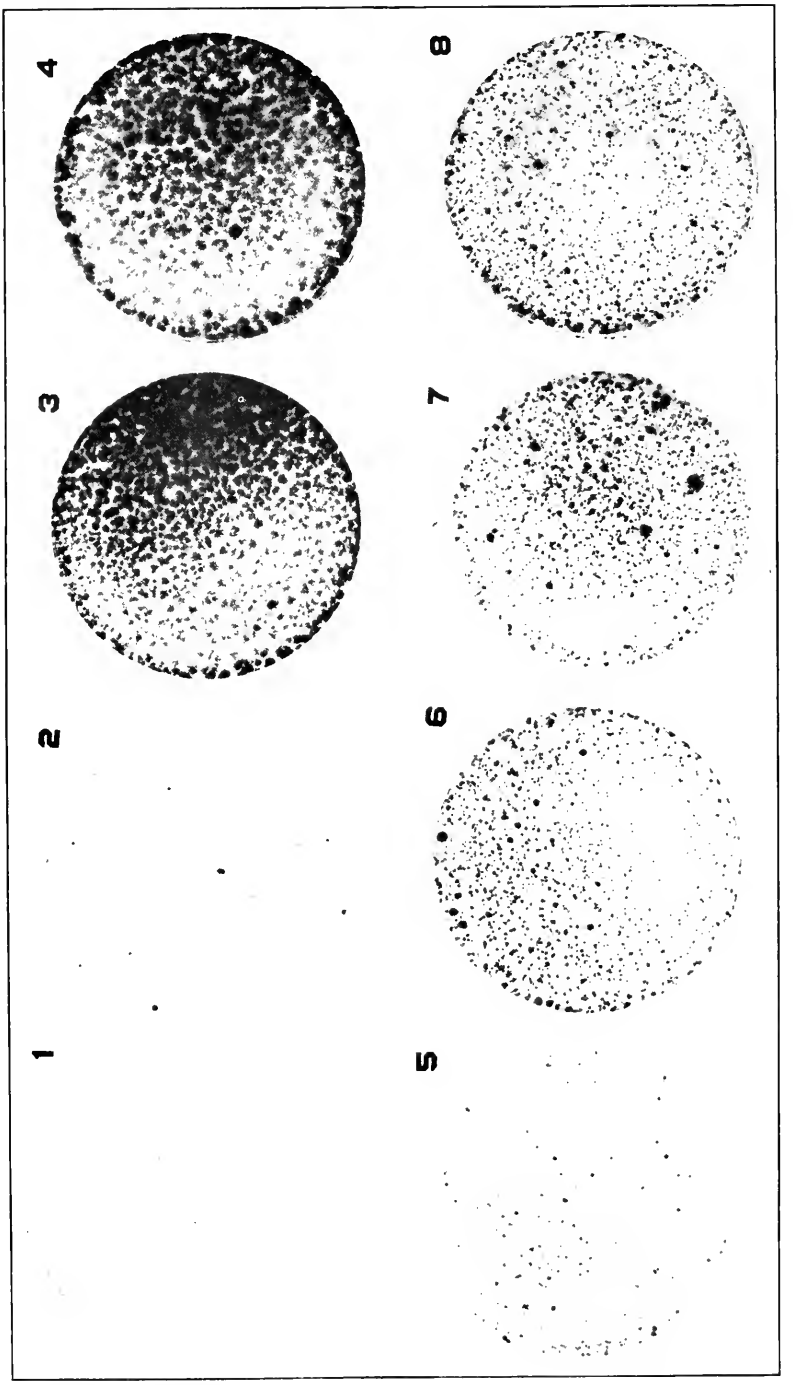
Fig. 2.—Photomicrograph of *S. atrovirens* in hanging-drop culture, showing development of conidiophore and conidia in mature stages.

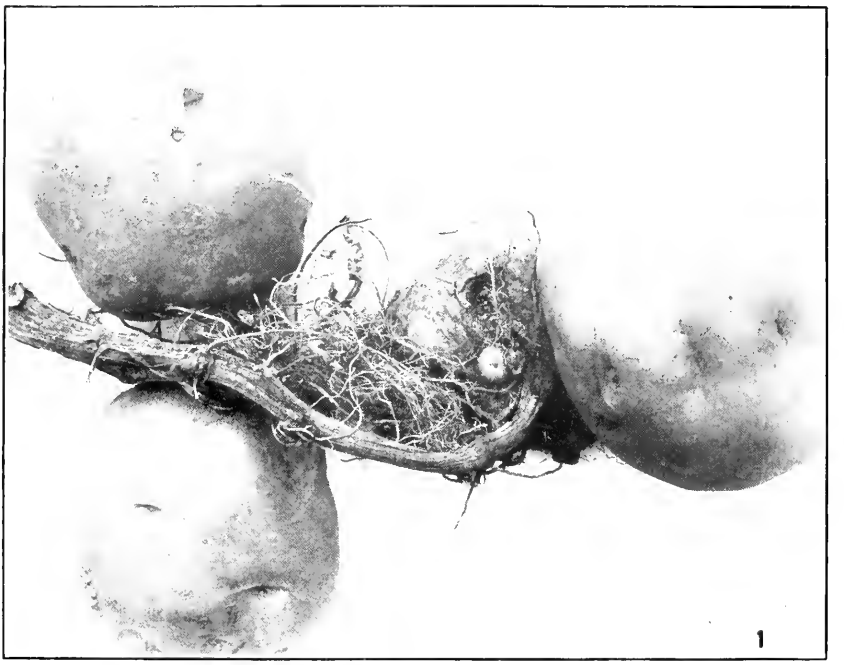
Fig. 3.—Negative heliotropism of *S. atrovirens* on corn-meal agar exposed on one side to daylight from April 8 to April 24, 1915, in laboratory at room temperature.

PLATE XLVII

Effect of temperature upon mycelial development of *Spondylocladium atrovirens* in pure culture on corn-meal agar at end of four weeks.

Petri Dish No.	Temperature (°C.)	Petri Dish No.	Temperature (°C.)
1	3	5	10
2	5	6	15
3	24	7	16
4	27	8	18


4
8
3
7
2
6
1
5


1

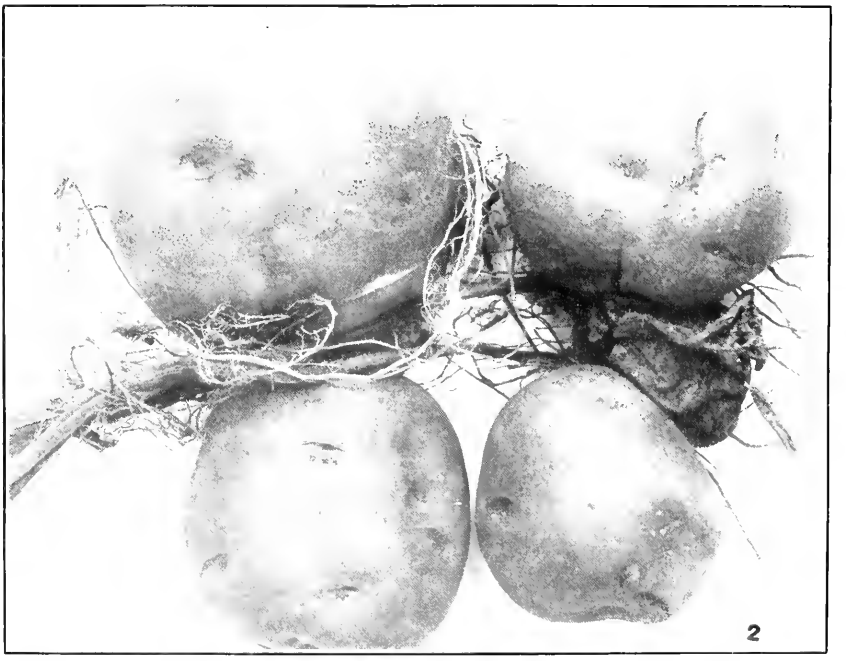

2

PLATE XLVIII

Contact infection. A part of the new tubers becoming infected with *Spondylocladium atrovirens* by means of contact with the infected mother tuber. In this case it is a distinctly stem-end infection. Harvested on September 19, 1915, at Presque Isle, Me.

37767°—16——2

WOOLLY PEAR APHIS[1]

By A. C. BAKER, *Entomological Assistant*, and W. M. DAVIDSON, *Scientific Assistant*, *Deciduous Fruit Insect Investigations, Bureau of Entomology*

INTRODUCTION

For some years a species of Eriosoma has been known to attack pear roots in California. It has, however, been considered to be the woolly apple aphis, *Eriosoma lanigerum* Hausmann, since both in habit and in structure the two species somewhat resemble each other. To the species on the pear, which, after careful study, proves to be undescribed, the name "*Eriosoma pyricola*" is herein given, and a brief account of the species is attempted.

HISTORY OF THE INSECT

Mr. Frank T. Swett is authority for the statement that the woolly pear aphis has been in California for more than 20 years. Ten years ago he says the species ruined about 2,000 French seedlings in one block, while occasional apple seedlings, planted along with them, made normal growth. Attention has frequently been called to the immunity of apple seedlings planted close to infested pear seedlings in nurseries and orchards.

During September and October, 1897, Mr. Theodore Pergande received specimens of a species of Eriosoma on pear roots from Prof. F. M. Webster, of Wooster, Ohio. Through the kindness of Mr. Pergande we have been able to examine these specimens, and they prove to be identical with our California material. It is quite possible, therefore, that the species may be present in other parts of the country, notably in Oregon. It is noteworthy that the Ohio specimens were taken from roots of pear stock received from France the preceding spring.

The species occurs over practically all the pear sections of northern and central California, and in some regions is a very destructive pest. To entomologists the extent of its presence has been known only for the last three or four years, but reports from orchardists and field observers indicate that it has been parasitic upon pear roots for a much longer period.

HABITS OF THE INSECT

The insect works entirely underground. The species that has been found feeding on the aerial portions of Nelis, Easter Beurré, and other pears is the woolly apple aphis, *E. lanigerum*. The woolly pear aphis

[1] What is probably the same species has been treated as a pear pest in California under the name *Eriosoma lanigera* by Geo. P. Weldon. (The woolly aphis as a pear pest. *In* Mo. Bul. State Com. Hort. [Cal.], v. 4, no. 9, p. 441–444, fig. 94–95. 1915.)

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
ea

Vol. VI, No. 10
June 5, 1916
K—33

appears to attack the roots of all types of pears, and it is especially injurious to the French wild stock so largely used in California as a stock for the Bartlett. Quince roots are fed upon, but much less freely, and the quince may be credited with a considerable degree of immunity. The Kieffer stock is attacked, but it is possible that Japanese stock may show immunity to a satisfactory degree. Observations to date indicate that both these stocks are more resistant than that from France. It should be said that the individual plants of the wild stock from France vary greatly, and there appears to be among the plants some variation in intrinsic vigor or in power to resist the woolly aphis. However, the majority of the imported seedlings show no satisfactory evidence of a power of resistance, and a different stock is very desirable.

The insect works especially upon the smaller fibrous rootlets and may be encountered on any such rootlets within the topmost 3 feet of soil and perhaps deeper. Infestations are usually heavier on the rootlets near the trunk, but frequently the aphides are as abundant 10 or 12 feet from the stem. In a badly infested orchard the soil on being overturned may in places be found to be white with the wool and skins of the insects. The aphides attack less frequently larger roots up to ¼ inch in diameter and sometimes settle on still larger roots or on the main stem where abrasions have set up a callus growth. They often colonize the underground portions of sucker growth, feeding on the succulent stalks. After the insects have forsaken a rootlet, fungi sometimes appear and complete its destruction.

This method of feeding upon the fibrous rootlets is somewhat analogous to the habits of the grape phylloxera (*Phylloxera vitifoliae* Fitch) on the resistant types of grapevines in that chiefly the smaller rootlets are attacked. It is directly opposed to the habits of the woolly apple aphis and of the grape phylloxera on nonresistant types of vines, for both these insects feed upon the larger roots and cause the formation of tuberlike lesions. The woolly pear aphis rarely forms any perceptible lesions, but it destroys great numbers of young rootlets, especially in late summer and autumn. In old trees this sometimes results in a dwarfing of growth and in a generally unthrifty appearance and condition. The majority of old infested trees do not show evident injury ascribable to the aphis, although it is presumable that they are suffering to some extent. They remain thrifty on account of their intrinsic vigor. In many instances where old trees were showing injury, extra cultivation of the soil and better irrigation practice resulted in the establishment of thrifty conditions, even though this method did not appear to reduce the numbers of the aphis. The effect on the crop is hard to estimate and can not be satisfactorily specified, but in general it is such as may result from the diversion of the flow of sap in the tree.

With trees under 4 years of age, conditions of injury are different. Heavy infestation of a tree of weak vigor or resistance may result in the death of the tree. Badly stunted growth and the early falling of foliage are characteristic of aphis injury on young trees. Injury and death are due to heavy summer and autumn infestations on the fibrous rootlets and to the inability of the tree to replace the destroyed roots quickly enough to afford plant food for the vegetative portion. Frequently the trees are saved and relief comes from the production in the fall months of a high percentage of migrants which leave behind them for the winter only a small infestation of wingless individuals; and since the aphides increase but slowly in spring, the tree is enabled to send forth new rootlets without danger of having them rapidly destroyed. Sometimes young trees in no wise stunted have been observed to cast their leaves prematurely, and upon examination have been found to be heavily infested with the aphis. It would appear from the absence of stunted growth that these trees did not have, or were not adversely influenced by, an infestation until their summer growth was about completed, and that the simultaneous destruction of feeding rootlets cut off the flow of sap suddenly. The fact that trees were stunted was an indication that the injurious effects of feeding by the aphides were felt earlier in the season.

In addition to trees noticeably stunted and others prematurely defoliated are found still others which show no external evidence of infestation and yet upon examination prove to be heavily infested. This phenomenon is frequently noticeable among young trees or in nursery rows, and hints at a power of resistance.

In orchards and districts where conditions favor large productions of winged forms, or migrants, spring and early summer infestations are small, denoting that few insects passed the winter on the roots. After the month of June, however, such infestations multiply rapidly and become very large by September, the month in which the fall migrants are produced in greatest abundance. After September there remain small wingless colonies which increase but little until the summer following. The winged forms are produced in abundance on heavy dry clay soils which crack in summer and autumn. Irrigated orchards produce them in smaller numbers than those that receive no moisture from May to October. On loam, silt, and light-clay soils the winged forms are much less abundantly produced. On such soils the infestation remains largely or wholly wingless the year around, and the conditions are generally unfavorable to such heavy infestations as occur on the heavy clays. The aphides appear to lack freedom of movement, and frequently their colonies are unable to increase perceptibly through summer. Occasionally the wingless infestations are severe the year round; where this is so, in the early part of the year there is caused a considerable stunting of growth and more or less

weakening, unless the trees can put out plenty of new rootlets to replace those injured and destroyed. This condition has been noted especially on light-clay soils where poor cultivation was employed.

SPREAD OF THE INSECT

In nurseries under favorable conditions the spread of the insect may be rapid. A half-acre pear nursery examined on June 9, 1915, failed to show infestation, though the aphis was probably present. When visited four months later, on October 16, it was found that more than half the trees examined were infested, some quite heavily. In large orchards where the soil is permeated throughout with rootlets the aphis doubtless is very easily diffused through the soil. In young orchards conditions indicate that not much spread takes place from tree to tree. Infested young orchards generally point to the nursery as the source of infestation, but the possibility of infestation through the winged forms, or migrants, must be considered. A knowledge of the full life cycle of the insect alone can clear up this point.

BIOLOGY AND DESCRIPTION OF THE INSECT

The wingless individuals live chiefly on the small rootlets and less frequently on roots and the underground portions of the sucker growth. They are always somewhat elongate and are for the most part pale yellowish red, but they may vary from a pale pink or yellow to deep red. They are rather sparsely clothed with long, curling, woolly, or cottony filaments, of which there are four or six on each segment. Toward the end of each instar these filaments are longer than the body—often three times as long. There is a sparse whitish powder on the body, more abundant at the caudal end. The cornicles appear as dusky-rimmed pores. The young are pale yellowish red and elongate.

The pupæ develop on the same portions of the tree as the wingless forms. They are very elongate in form and are clothed as are the wingless. The wing pads are inconspicuous and are white or light gray. As a rule pupæ on a rootlet develop almost simultaneously. The winged forms issue together, after which the narrow, elongate, cast pupal skins are conspicuous in little heaps, and are easily distinguishable from those of the wingless forms.

In the Walnut Creek district pupæ and winged migrants were collected in appreciable numbers from August 25 to November 17, and as late as December 22 a nymph was found. These forms were most abundant in September, and this observation apparently holds true for other localities in California. Wingless colonies collected at San Jose, Cal., on June 10 and thereafter, kept in Petri dishes with moist sand in a cellar, produced pupæ on July 20 and migrants from July 24 to August 7. This appeared to be abnormally early in the year for the production of winged forms, and

it may be that the environment and conditions hastened it. Under favorable conditions of soil the migrants were produced in great abundance on both young and old pear trees. In many cases, especially on young trees, it appeared that fully 90 per cent of the aphides observed at one time were pupæ, and in other instances observations in October and later after the winged forms had departed indicated that almost the entire infestation had developed into migrants. On old trees there remained on the average a larger residue of wingless forms. On unfavorable types of soil the winged forms are produced in far less abundance. It appears to be a rule that the heavier and drier the soil the larger the percentage of pupæ developing. It sometimes happens that the migrants are unable to rise to the surface of the ground and become imprisoned in pockets in the soil. In one instance two living sexual females were found in such a pocket beside dead migrants.

The winged forms have been noticed on pear foliage and on the trunk, but with one exception [1] no deposition of sexes has been observed on the pear. On cork and American elms (*Ulmus* spp.) migrants were observed to deposit the sexes in cracks in the bark and on the lower surface of leaves. In one instance the migration from a nursery of pear trees to a group of young elms 200 yards distant could be traced. The migrants fly readily and strongly and are stimulated by the sun's rays, being more active on warm than on cool days. On the elms they were more abundant on trees with rough bark than on the smooth-barked plants.

The migrants vary considerably in size. They are rather elongate, shining black or dark green, with a tuft of white wool on the caudal segment; otherwise, there is no flocculence. The lower surface is dark green, sparsely powdered at the sutures. The antennæ, eyes, and a portion of the legs are black. The base of the femora and the middle portion of the tibiæ are yellowish brown or amber. The wings have narrow black veins and a greenish blue stigma. The wing insertions are sometimes brown, but are more often yellowish. In recently molted individuals there is sometimes a smoky-brown patch at the base of the fore wings.

To obtain the sexes, migrants were confined in stender dishes and in small rubber cells mounted on microscope slides with cover glasses as lids. Some were kept in a lighted room in which the temperature varied very considerably, at times rising to 75° and at other times falling to 55° F. Others were kept in a dark cellar where the temperature varied but little and averaged about 61° F. Under cellar conditions the migrants deposited more sexual forms than under the conditions obtaining in the room. Some of the dishes were kept dry and others moistened to different degrees. In the moistened dishes the sex pro-

[1] In August, 1911, at San Jose, Cal., a migrant was noticed depositing sexes on the upper surface of a pear leaf.

duction was better than in the dry ones, although too much moisture prevented the sexual forms from freeing themselves from the pellicles. Whether the migrants had flown or not did not seem to bear any influence on the deposition of the sexual forms. In most of the dishes more than half of the sexed forms were not extruded, but died unborn. In the rubber cells five-eighths of an inch in diameter and three-sixteenths of an inch in height the migrants did best singly, while the larger stender dishes provided space for a number. In all the dishes pieces of pear or elm bark were provided, but the migrants rarely deposited the sexes on these, nearly always extruding them on the filter paper also provided. It frequently happened that the sexes after having been extruded became entangled with the wings or legs of the parents or with each other. The sexes were deposited in rapid succession. The migrants rarely lived beyond three days after they were placed in the dishes, whether they deposited sexual forms or not. None lived longer than six days. They died immediately after the sexes had been extruded and very few deposited their full complement.

All the sexes deposited were not noted; but about four-fifths of them totaled 109 individuals, of which a little over half (58) were females. Only a few matured, and the majority died unmolted. Undoubtedly the cause of this was the abnormal condition of the environment. However, it appears to be proved that the sexes are produced in about equal numbers, and observations in the field corroborate this. Four fall migrants dissected on October 27 and 28 had contained, respectively, 5, 7, 8, and 9 young. In the dishes not more than seven sexes were ever dropped by an individual. The number of males and females deposited by individual migrants was found to range from seven females and no males to five males and one female. Probably a larger series would have furnished a migrant producing only males. As a rule the production of sexes was about evenly divided between male and female.

The sexes have no woolly covering such as that occurring on the sexes of *Eriosoma lanigerum*, but are bare and shining. The female, however, at the time of depositing the winter egg, has a patch of short white wool on either side of her body and with this she contrives to clothe partly the winter or impregnated egg. The sexes are active, the male especially so, both immediately after extrusion and following the casting of their fourth and final skin. Between casting their first and fourth skins they remain inactive unless disturbed. Normally they seek crevices in the bark, but in the dishes they frequently molted on filter paper or on the sides and floor.

The sexes mature in from 7 to 11 days and molt four times—that is, about every other day. Being beakless, they take no food.

The males are smaller than the females, the latter being enlarged by reason of the egg within the body. The male at first is light green, with

hyalin antennæ and legs and black eyes of three facets. The insect becomes darker with age and the mature individual is dark olive-green, sometimes tinted with lilac or purple, the central part of the abdomen being darkest. The male is always narrow in shape. The female varies in color from a light orange to a dark red. The eyes and appendages are as in the male. The majority are orange or a light crimson-lake. They are much stouter than the males and are longer and stand much higher. A mature female measured alive was 0.67 mm. long by 0.33 mm. in maximum width. A mature male was 0.43 mm. long by 0.21 mm. in maximum width.

Copulation occurs as soon as the sexes are mature. It appears that unless the female is fertilized directly after she has cast her last skin she will fail to deposit the winter egg. The male may live at least a week after he is mature, but apparently he can exercise the sexual function only immediately after he has cast the last skin. The females deposit the impregnated egg immediately after copulation, and after its deposition they may live for a day or two at the most. The winter or impregnated egg is laid normally in crevices or scars of the bark of the elm. In the dishes it was laid sometimes on the outside of the bark, and both elm and pear bark were used. It was never laid elsewhere than in the bark. The egg measures about 0.444 mm. by 0.225 mm., is short oval, reddish yellow, and shining. The end first extruded is reddish and bare, while the other extremity is yellowish and usually covered with short white wool provided by the female. Winter eggs were deposited in dishes between October 15 and November 12. Undoubtedly they occur in nature as early as September 5, and may be laid as late as the middle of November. Toward the end of October some were collected under the bark of elms under observation. Table I is a comparison of the biology of *Eriosoma pyricola* with that of *E. lanigerum*.

TABLE I.—*Comparison of biology of Eriosoma pyricola with that of Eriosoma lanigerum in California*[1]

Eriosoma lanigerum on apple and varieties of pear.	*Eriosoma pyricola* on pear.
Aerial and radical.	Radical only.
Attacks trunks, branches, and twigs; causes knotty swellings on roots.	Attacks chiefly fibrous rootlets; rarely causes lesions; occasionally settles on larger roots.
Fall migrants rarely abundant; apparently not influenced by conditions.	Fall migrants very abundant under favorable conditions.

[1] The full cycle of these species has not been worked out in California, but there appear to be no records of spring generations of *E. lanigerum* observed on elm.

The fall migrants of *E. pyricola* may be distinguished from those of *E. lanigerum* and *E. americanum* as shown in Table II.

TABLE II.—*Comparison of the fall migrants of Eriosoma pyricola, E. lanigerum, and E. americanum*

E. pyricola.	*E. lanigerum.*	*E. americanum.*
Stigma short, greenish blue.	Stigma somewhat elongate, yellowish or gray.	Stigma elongate, gray.
Veins narrow without brown margins.	Veins narrow and without brown margins.	Veins broad, with brownish margins.
Body naked except for caudal segment.	Body with some woolly clothing.	Body with slight woolly covering.
Distal sensoria of antennal segments V and VI with fringe.	Distal sensoria of antennal segments V and VI without fringe.	Distal sensoria of antennal segments V and VI without fringe.

The new species is easiiy distinguished from *E. ulmi* Linnæus from the fact that segment V bears prominent transverse sensoria. The wingless forms can be distinguished from those of *E. lanigerum* by the structure of the compound wax pores, and the winged forms by the antennæ. The winged forms of *E. pyricola* are remarkably like those of *E. lanuginosa* Hartig. The proportions are almost exactly the same. The only difference seems to be the fringing of the sensorium on segment V. The wingless forms and the pupæ have the prominent wax pores figured. No such pores occur in our specimens of *E. lanuginosa*, but very similar ones do occur in *E. ulmi*. At first it was thought that two species were present in the collected material, but careful rearing experiments by the junior writer have shown the connection between all the forms. It does not seem probable that such prominent wax-secreting structures would be present in one form of the species and not in all forms.

Eriosoma pyricola, n. sp.

WINGLESS VIVIPAROUS FEMALE.—General form elongate. Antennal segments in length as follows: I, 0.048 mm.; II, 0.048 mm.; III, 0.1 mm.; IV, 0.04 mm.; V, 0.048 mm.; VI, 0.064 mm. (unguis, 0.032 mm.); segments armed with hairs (fig. 1, *E*), which are considerably longer than those met with in *lanigerum* (fig. 1, *D*), and with a large distal fringed sensorium on segments V and VI, as well as some smaller ones on VI. Compound wax pores very prominent and circular (fig. 1, *I*), those on the abdomen containing about 20 cells. Abdomen sparsely covered with hairs about 0.16 mm. long; cornicles circular, their rims more heavily chitinized on their inner margins than elsewhere. Wax reservoir apparently present as in *E. lanigerum* (visible as a clear yellow area in mounted specimens). Hind tibiæ about 0.44 mm. long; hind tarsus, 0.112 mm.; rostrum extending beyond the second pair of coxæ. Length, 1.92 mm.; width, 0.96 mm. The hairs on the antennæ of the young are especially prominent (fig. 1, *J*).

Young forms yellowish pink, older ones pink to red. Antennæ, legs, and labium dusky; eyes dark red, very minute.

INTERMEDIATES.—In the collection, Q. 6399, are a number of specimens which would be taken at first glance for wingless viviparous females. A careful study, however, proves them to be intermediates. No trace of wing pads can be found, but the eyes clearly show the intermediate nature of the specimens. In the normal wingless

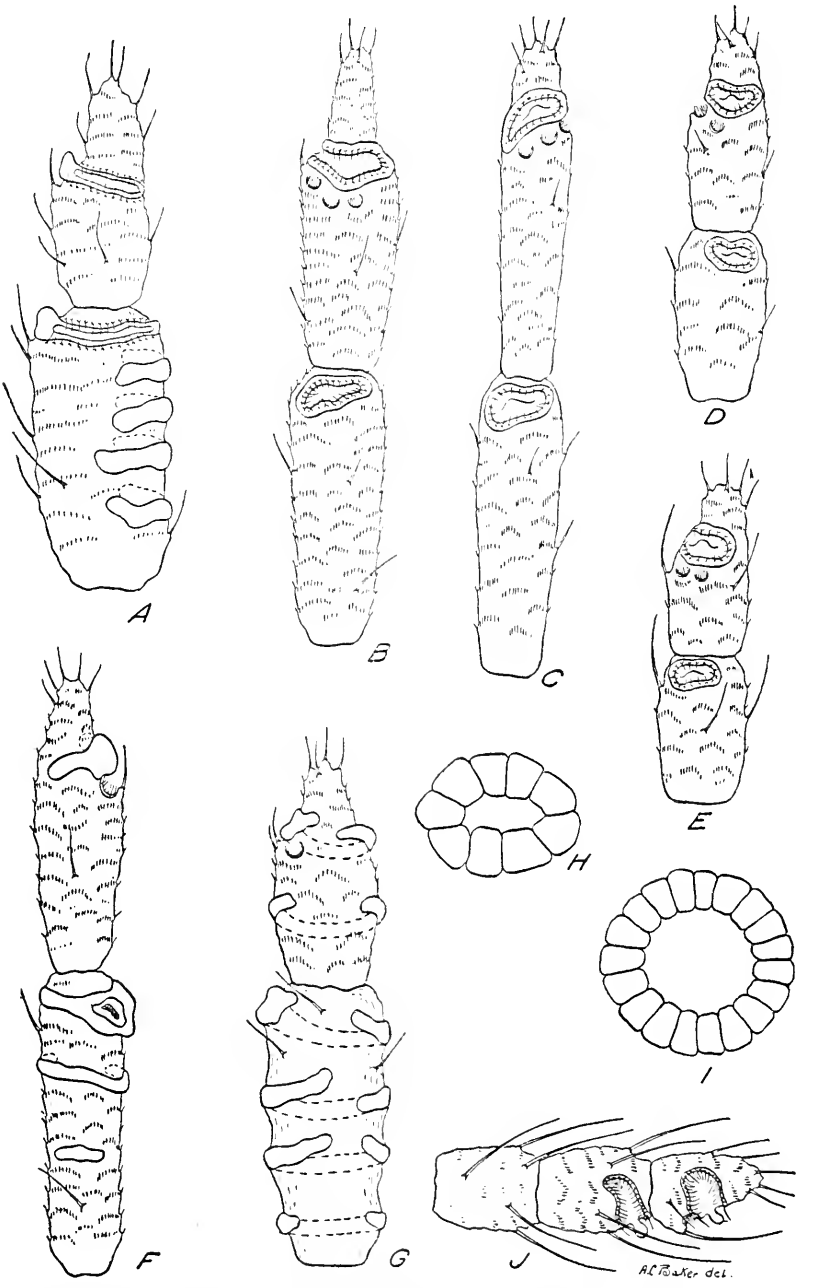


FIG. 1.—Comparative structure of antennæ and wax pores of *Eriosoma* spp.: *A*, distal segments of antenna of winged viviparous female of *E. pyricola*; *B*, distal segments of antenna of winged viviparous female of *E. ulmi*; *C*, distal segments of antenna of wingless viviparous female of *E. americanum*; *D*, distal segments of antenna of wingless viviparous female of *E. lanigerum*; *E*, distal segments of antenna of wingless viviparous female of *E. pyricola*; *F*, distal segments of antenna of winged viviparous female of *E. americanum*; *G*, distal segments of antenna of winged viviparous female of *E. lanigerum*; *H*, compound wax pore of *E. lanigerum*; *I*, compound wax pore of *E. pyricola*; *J*, distal segments of antenna of first instar wingless viviparous female of *E. pyricola*.

forms the eyes are composed of three facets and are very minute, whereas in these specimens the eyes are large and composed of numerous facets, thus approaching the compound eyes of the winged form. All other characters met with are those of the wingless viviparous female.

PUPA.—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.192 mm.; IV, 0.064 mm.; V, 0.08 mm.; VI, 0.08 mm.; segments armed with hairs and sensoria as in the wingless female. Wing pads about 0.64 mm. long. Compound wax pores similar to those of the wingless females. Hind tibia, 0.432 mm.; hind tarsus, 0.128 mm. Body with long hairs as in the wingless form. Length, 2.32 mm.; width, 0.96 mm.

Pinkish, with a brick-red diffusion; wing pads whitish yellow; wool sparse, erect.

WINGED VIVIPAROUS FEMALE (FALL MIGRANT).—Antennal segments in length as follows: I, 0.048 mm.; II, 0.064 mm.; III, 0.432 mm.; IV, 0.112 mm.; V, 0.112 mm.; VI, 0.08 mm. (unguis, 0.032 mm.); segments I and II armed with a few hairs; segment III armed with about 20 transverse sensoria, which extend a little over halfway around the segment as in *E. lanigerum*, the dorsal side of the segment armed with numerous prominent hairs; segment IV similar to segment III and armed with four or five transverse sensoria; segment V (fig. 1, *A*) armed with three or four transverse sensoria and a distal fringed sensorium, a few hairs, and many rows of setæ; segment VI similar to segment V, but without transverse sensoria. The fringed sensorium at the base of the unguis varies in shape. Forewing somewhat similar to that of *E. americanum;* stigma short and rounded at the distal extremity. Hind tibia, 0.88 mm.; hind tarsus, 0.128 mm. Form elongate; length, 1.76 mm.; width, 0.72 mm.; forewing, 2.4 by 0.88 mm. Without wool.

Dark brown or very dark green. Base of femora and tibiæ yellowish gray. Stigma bluish gray. Abdomen shining.

Described from wingless females, intermediates, pupæ, and winged viviparous females in balsam mounts.

Type: Cat. No. 20083, U. S. National Museum.

PATHOLOGICAL HISTOLOGY OF STRAWBERRIES AFFECTED BY SPECIES OF BOTRYTIS AND RHIZOPUS

By NEIL E. STEVENS,
Pathologist, Fruit Disease Investigations, Bureau of Plant Industry

INTRODUCTION

The fungi causing rots of strawberries (*Fragaria* spp.) in transit from the Southern States have been under investigation by Dr. C. L. Shear, Mr. R. B. Wilcox, and the writer for the past two years. From the first it has been apparent that two species were chiefly responsible for their decay during shipment and on the market. These were *Botrytis* (*cinerea*?) and *Rhizopus* (*nigricans*?).[1] The effect of these two fungi on ripe strawberries is strikingly different. Berries injured by *Botrytis* sp. show a characteristic dryrot—that is, they retain their shape, shrivel somewhat, and no leaking of juice is evident; whereas berries rotted by *Rhizopus* sp. quickly flatten out, with the loss of a large amount of juice. Such berries are characterized as "leaks" by growers and dealers.

F. L. Stevens[2] has already recognized a species of Rhizopus as the probable cause of leak. He, however, considers (p. 950) that *Botrytis* sp. "is the primary cause of the molding, that the *Botrytis* initiates the decay, opening the way to such other saprophytes as may be present; of such saprophytes, *Rhizopus* is by far the most prominent and most abundant." In order to determine if possible the relations of these fungi in rotting strawberries and in particular what differences exist in their method of attack on the fruit, a study of strawberries affected by these fungi was undertaken.

EXPERIMENTAL METHODS

The strawberries examined were chiefly of the Klondike variety grown in Louisiana during the season of 1915. Berries of other varieties grown in South Carolina and at Arlington Experimental Farm, Va., in 1915, as well as the Missionary and Klondike varieties from Florida in 1916, were used for comparison. Naturally infected berries as well as sound berries inoculated with spores and mycelium from pure cultures were used in both cases.

The material was fixed in a solution of equal parts of absolute alcohol and glacial acetic acid. This fluid penetrates very rapidly, so that whole strawberries are satisfactorily fixed. In the case of large berries,

[1] In the present uncertainty regarding the taxonomy of these genera it seems unwise to attempt a definite determination of the species. Permanent mounts of the material described are preserved, however, and cultures of the species considered are retained for further study.

[2] Stevens, F. L. A destructive strawberry disease. *In* Science, n. s., v. 39, no. 1017, p. 949–950. 1914.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dz

Vol. VI, No. 10
June 5, 1916
G—82

however, the ends were cut off to hasten penetration. Strawberry cells are so large that rather thick sections, from 10 to 20μ, were found most desirable. The walls of the strawberry cells and of the fungus hyphæ are so similar that differential staining was rather difficult. The best differentiation was secured by a combination of methylene blue and clove-oil eosin, using a water solution of tannin as a mordant. This method was suggested to the writer by Mr. Charles S. Ridgeway, of the Bureau of Plant Industry. The hyphæ, however, are so large as to be easily distinguished when the sections are properly stained with the more permanent stains, as safranin, Delafield's hematoxylon, or even Bismarck brown.

RESULTS OF INFECTION OF STRAWBERRIES BY BOTRYTIS SP.

Botrytis sp. has long been a favorite subject for the investigation of the relations of host and parasite. The somewhat conflicting views held by different investigators as to the nature of its attack on the host are well summarized by Brown [1] in a recent paper. In general, all writers agree on the presence of a cell-wall dissolving enzym, but differ widely as to the cause of the toxic action of the fungus.

As already stated, strawberries rotted by *Botrytis* sp. retain their shape, shrivel slightly, and even in a moist chamber there is no evident leaking. The moisture is apparently lost so slowly that it evaporates from the surface of the berry. A microscopic examination shows that the fungus has penetrated all parts of the berry; indeed, the cells are in many places embedded in the mass of mycelium and are apparently held together by it. The fungus is evidently capable of readily dissolving the middle lamella and of penetrating the cell walls themselves. Often hyphæ grow between the cells of the host for some distance and then penetrate the cells (Pl. XLIX, *A*). Not infrequently cells containing numerous hyphæ have the shrunken and distorted protoplasmic contents still present (Pl. XLIX, *B*, *C*, *D*). Sometimes hyphæ occur in adjacent cells whose separating wall remains intact and apparently unchanged (Pl. XLIX, B); or they may pass from one cell into the next, either where the cells are in contact or across an intercellular space (Pl. XLIX).

It is interesting to observe that hyphæ usually enter a cell at the angle where it joins two other cells; Plate XLIX, *D*, *F*, and *G*, shows examples. The hypha passes between two cells, apparently by dissolving the middle lamella, and then penetrates the wall of the cell with which it comes in direct contact. Occasionally a hypha seems to push back a portion of the cell wall before penetrating (Pl. XLIX, *G*). The fungus may, however, penetrate the wall at a considerable distance from the intersection of the cells (Pl. XLIX, *A*, *E*); or it may

[1] Brown, William. Studies in the physiology of parasitism. I. The action of Botrytis cinerea. *In* Ann. Bot., v. 29, no. 115, p. 313-348. 1915.

pass the point of intersection and penetrate a short distance beyond (Pl. XLIX, *H*).

Brown,[1] working with thin disks of tissue cut from various plants, particularly tubers of the potato and roots of the turnip, immersed in a strong extract from the germ tubes of *Botrytis cinerea*, noted that the separation of the cells followed the line of the cell walls, the cells on either side being left intact. His idea of the destruction of the cells is that the middle lamella is first dissolved, in consequence of which the tissue readily falls apart along the line of the middle lamella. Very soon the remainder of the cell wall disintegrates and the whole structure becomes very fragile.[2] In no case was complete solution of the cell wall observed. Death of the cells[3] takes place at a late phase in the process of disorganization of the cell walls. He observed also that in all cases if a cell wall was disintegrated death of the cell ensued; on the other hand, if the cell wall was not affected neither were the living contents of the cell.[4]

Brown's conclusions satisfactorily explain the condition found by the writer in strawberry cells attacked by *Botrytis* sp. Certainly the fungus is able to penetrate the cells of the host while they are still fairly normal in appearance and while the cytoplasm is still distinguishable (Pl. XLIX, *B*, *D*, *G*). The writer did not find, however, in any of the strawberries examined cells which were unaffected by the action of the fungus.

RESULTS OF INFECTION OF STRAWBERRIES BY RHIZOPUS SP.

In contrast to the condition of strawberries rotted by *Botrytis* sp., berries rotted by *Rhizopus* sp. show the following characteristics. The berries soon become flattened, with considerable loss of juice. Microscopic examination shows that the hyphæ are characteristically close to the surface of the berry, the majority being found in the outer six or eight cell layers. Hyphæ rarely or never penetrate the cells of the berry under field conditions or when kept in moist chamber. The nuclei of the cells persist in apparently normal condition until the cytoplasm of the cell has almost entirely collapsed.

The crowding of the fungus in the outer portion of the berry is very noticeable. Indeed hyphæ frequently grow for some distance immediately beneath the epidermis. Plate XLIX, *I*, shows a portion of such a hypha in a section cut nearly tangential to the surface of the berry. The small, thick-walled cells (heavy lines) on the right are epidermal cells; the larger, thin-walled cells (light lines) on the left are storage cells. The hypha, which could be traced across several sections, grows between these two layers of cells for a considerable distance without penetrating either. A similar condition is shown in vertical section in Plate XLIX,

[1] Brown, William. Op. cit., p. 333.
[2] Ibid., p. 335.
[3] Ibid., p. 347.
[4] Ibid., p. 345.

K, L. In the latter case the fungus has penetrated the epidermis and the external hyphæ are sporangiophores.

It is evident from a study of the sections that *Rhizopus* sp. does not readily penetrate the unbroken epidermis from the outside. Hyphæ are found which extend for some distance along the surface of the berry without penetrating. Plate XLIX, *J*, shows a portion of such a hypha; even the germ tubes seem unable to penetrate readily and often grow for some distance (Pl. XLIX, *M*) over the surface without penetrating.

Under field conditions or in moist chamber in the laboratory *Rhizopus* sp. apparently very rarely enters the host cells. Although several hundred slides were examined no single instance was found in which a hypha had penetrated a cell wall. Plate XLIX, *I–L*, shows that the hyphæ typically grow between the cells along the middle lamella. The effect of the fungus on the host cells is readily seen by the contraction of the protoplasm. Plate L shows strawberry cells in various stages of degeneration close to hyphæ of *Rhizopus* sp.

Plate L, *A*, shows the normal appearance of one of the smaller storage cells of the strawberry. In this case the cytoplasm contains numerous small vacuoles. Frequently, especially in larger cells, there is a single large vacuole. Plate L, *B*, shows a similar cell in which the protoplasm has begun to contract away from the wall. This cell was separated from the nearest hyphæ by three layers of cells. In Plate L, *C*, hyphæ of *Rhizopus* sp. are shown in contact with two host cells (a branch hypha overlies one cell). The protoplasm of these cells is much shrunken, but the cell walls retain their normal position, and the nuclei are unchanged. Plate L, *D*, *E*, *F*, and *G*, show progressively later stages in the breaking down of cells adjoining hyphæ. In some (Pl. L, *D*, *F*) the wall has begun to collapse. In all except Plate XLIX, *G*, in which there was very little cytoplasm remaining, the nucleus shows no signs of degeneration.

This persistence of the nucleus in apparently normal condition after the contraction of the protoplasm has progressed considerably is one of the most striking characteristics in berries attacked by *Rhizopus* sp. and is in sharp contrast to the condition found in berries rotted by *Botrytis* sp. Often in a cell in which the cytoplasm has largely disappeared and the wall is partly collapsed the nucleus appears large and typical, as in an intact cell (Pl. L, *J*). Frequently the cell wall collapses so rapidly that no space is left between it and the contracted protoplasm (Pl. L, *H*, *I*).

EFFECT OF RHIZOPUS SP. ON BERRIES IN EXTREMELY DRY AIR

In connection with experiments on the humidity relations of the fungus, berries inoculated with *Rhizopus* sp. were placed in a desiccator with concentrated sulphuric acid. Under these extremely dry conditions the berry "leaked" in the characteristic manner, but the habit of growth of the fungus was changed in two important particulars.

Fungus hyphæ were found in all parts of the berry, being abundant even in the center, within the circle of vascular bundles. Apparently the extreme dryness of the surrounding air made the intercellular spaces within the berry more favorable for its growth than the outer ones. Under these severe conditions the cells of the berry collapsed so generally that the relations of the fungus hyphæ to the walls could usually be studied only in cells near vascular bundles. It was evident that while, in general, the hyphæ grew between the cells of the host (Pl. L, *L*) they were frequently found inside the cells as well (Pl. L, *K*, *M*). It is worthy of note that in these berries several instances were found where hyphæ had punctured the cells and the nucleus of the cell was unchanged in appearance (Pl. XLIX, *K*).

COMPARISON OF THE FUNGI

The difference in the histological relations of the two fungi with the strawberry may be briefly summarized as follows:

Botrytis sp. penetrates all parts of the berry, growing within the cells as well as between them and ramifies through the tissues of the strawberry, surrounding and filling them with a network of mycelium. The cells of the berry seem to be quickly killed by the fungus; at least the protoplasm shrinks away from the cell wall and becomes disorganized so that no nucleus can be distinguished.

The mycelium of *Rhizopus* sp., on the other hand, is found chiefly in the outer portion of the berry. The hyphæ grow between the cells, separating them and apparently extracting the cell sap. The nuclei of the cells persist unchanged until a late stage in the breaking down of the cytoplasm. When the fungus is grown on berries in a dry atmosphere, its action is somewhat different. The mycelium penetrates to the center of the berry, and hyphæ are frequently found inside cells.

It is difficult to trace an exact causal relation between the histological differences in the attack of these fungi on the strawberry and the fact that they cause quite different types of rot. The fact that *Rhizopus* sp. separates the cells of the berries so completely may readily account for the berries affected with this fungus becoming so soft and easily flattened. On the other hand, the mycelium of *Botrytis* sp., by penetrating all parts of the strawberry, helps to hold it in shape and converts it into a mummy. It is possible that the juice of the berries affected by *Rhizopus* sp. is pressed out by the collapse of the berries, owing to the mere separation of the cells. This is, however, hardly an adequate explanation of the phenomenon.

While it is not proposed at the present time to review the rather voluminous literature on either of the fungi under consideration, a closely parallel case described by Behrens [1] should be mentioned in this

[1] Behrens, Johannes. Beiträge zur Kenntnis der Obstfäulnis. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 4, No. 12, p. 515-516. 1898.

connection. He observed in 1895 ripe tomatoes affected by *Mucor stolonifer* which reduced the pulp of the tomato to an almost fluid mass. A species of Fusisporium found at the same time on the tomatoes produced a dry-rot quite in contrast to the wet condition produced by the species of Mucor. Behrens found on microscopic examination that the mycelium of *Fusisporium* sp. penetrated the cells of the host, while the mycelium of *Mucor stolonifer* grew entirely in the intercellular spaces.

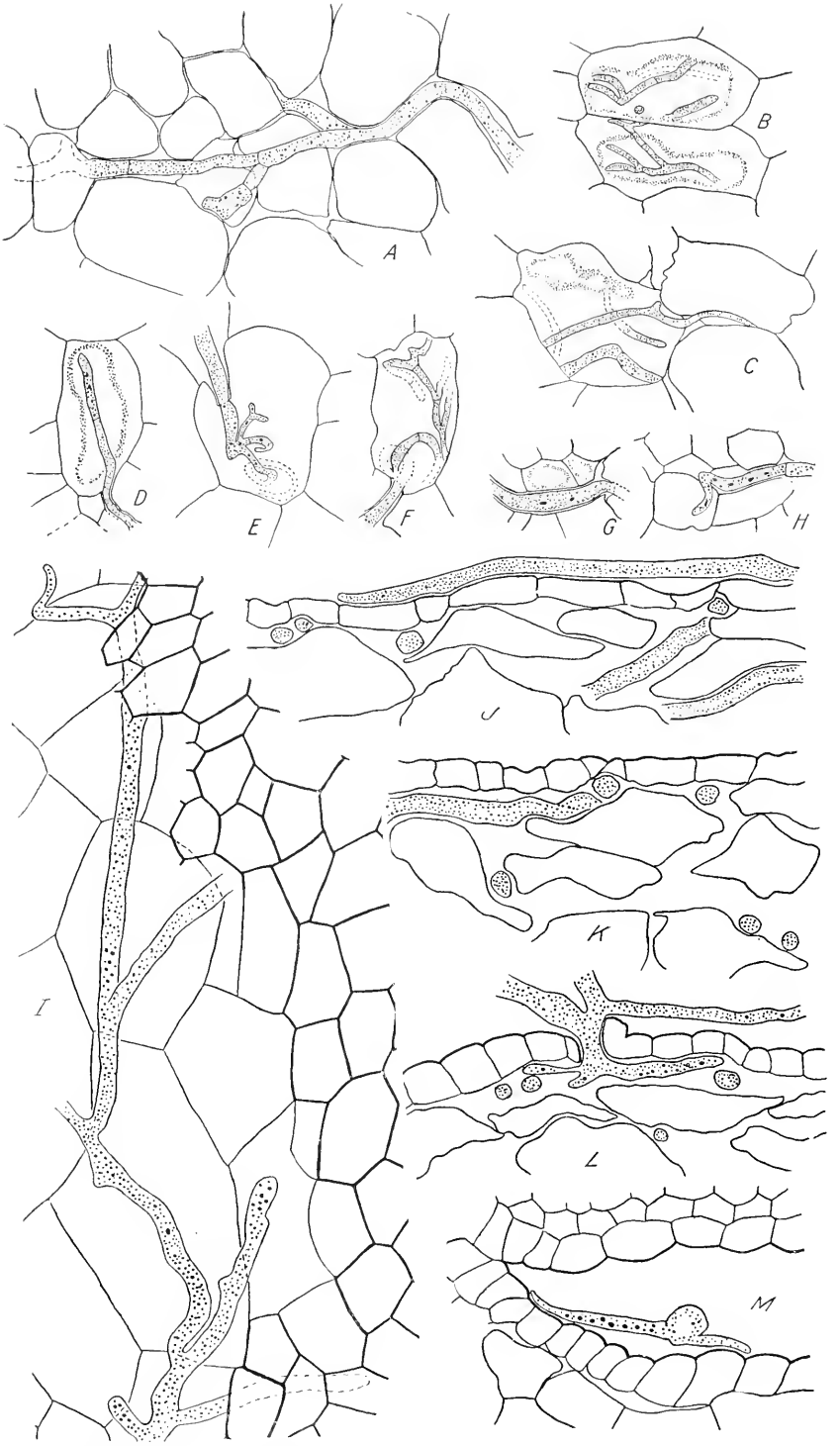
The relation of these fungi to each other in their attack on the berry is much clearer. In comparatively few cases have both fungi been found on the same berry and in no instance has the writer found a berry in which *Rhizopus* sp. had followed in a place originally infected by *Botrytis* sp.

Numerous cases have, of course, been found in which there were two fungi in the same berry; for instance, *Botrytis* sp. and *Fusarium* sp., *Botrytis* sp. and *Alternaria* sp., *Rhizopus* sp. and *Fusarium* sp. These fungi do not, however, seem to have entered in the same place, but rather from different portions of the berry. The mycelia of the two fungi sometimes mingle in the tissues of the berry—for example, *Botrytis* sp. and *Fusarium* sp., *Rhizopus* sp. and *Fusarium* sp.—or they may occupy different portions of the berry with a marked line of division between them, each apparently being unable to invade tissue occupied by the other fungus—for example, *Botrytis* sp. and *Alternaria* sp.

These observations do not preclude the possibility of *Rhizopus* sp. following in an area originally infected by *Botrytis* sp. or some other fungus, and this may occur in the field or in badly affected berries which are thrown out as culls in packing. They do, however, plainly indicate that *Rhizopus* sp. is not dependent on the presence of any other fungus in its attack on strawberries during shipment and on the market.

PLATE XLIX

A–H, Strawberry cells attacked by *Botrytis* sp. (× 210): *A*, Hypha growing partly between and partly within strawberry cells; *B*, hyphæ inside strawberry cells in which remnants of the protoplasm may still be distinguished; *C*, hypha passing from one cell into another across a short intercellular space; *D*, *E*, *F*, *G*, *H*, hyphæ entering cells in various ways (in *G* the hypha has pushed back a portion of the cell wall before breaking through). *I–M*, Strawberry cells attacked by *Rhizopus* sp. (× 210): *I*, Hypha growing between the epidermis and the adjacent layer of storage cells; *J*, hypha growing over the surface of the strawberry; *K*, hyphæ growing underneath the epidermal layer and between the storage cells; *L*, *Rhizopus* sp. growing between epidermal cells (basal portions of sporangiophores above and rhizoids below epidermis); *M*, germinating spore in cavity formed by a seed.


A
B
C
D
E
F
G
H
I
J
K
L
M

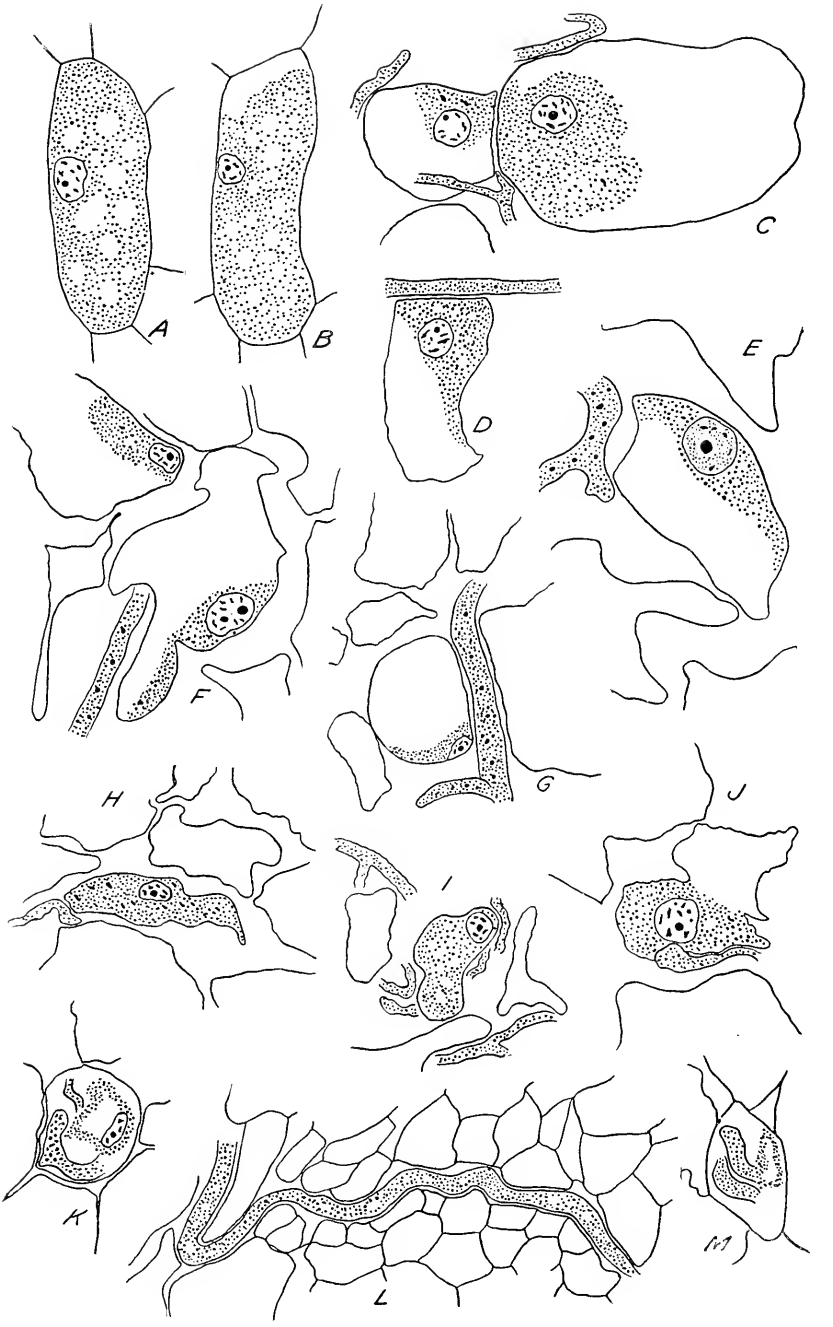

A
B
C
D
E
F
G
H
I
J
K
L
M

PLATE L

Strawberry cells attacked by *Rhizopus* sp. *A*, Normal storage cell of strawberry; *B*, storage cell (near hyphæ) showing a slight contraction of the protoplasm; *C*, *D*, *E*, *F*, *G*, progressive contraction of protoplasm of host cells near hyphæ (the cell walls have contracted very little); *H*, *I*, *J*, strawberry cells near hyphæ in which the cell wall has crumpled with the contraction of the protoplasm; *K*, *M*, hyphæ inside cells; *L*, hyphæ growing between cells of the strawberry; *K*, *L*, *M* are drawn from berries which had been rotted in the desiccator. (× 210.)

LIFE HISTORIES AND METHODS OF REARING HESSIAN-FLY PARASITES

By C. M. PACKARD,[1]
Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology

INTRODUCTION

The most effective factors in the control of the Hessian fly (*Mayetiola destructor* Say) in the past have been its parasites. There are seasons, however, when the parasites become scarce and the Hessian fly exceedingly abundant. Again, in the same season the Hessian fly seems practically free from parasites in some localities while in others its parasites are numerous. A thorough knowledge of the life histories, field habits, relative efficiency, and effective methods of artificial propagation and dissemination of the different parasites, therefore, might make it practicable to introduce the most efficient species from localities where they are abundant into other localities where the host is working destruction unchecked by its enemies. It might also be possible to propagate artificially and to disseminate the parasites during periods when they have become scarce in the fields, and thereby shorten the period of destructive abundance of the Hessian fly. Up to the present time very little accurate and detailed information seems to have been recorded regarding the life stages, habits, and efficiency of Hessian-fly parasites. It has been uncer tain whether or not some of the species involved were true parasites. Some results in this direction have been accomplished by the author during the last two seasons, and the purpose of this paper is to make public these results and the methods used in attaining them.

The life histories and methods of rearing three hymenopterous parasites are treated in this paper: *Eupelmus allynii* French, *Merisus destructor* Say, and (*Merisus*) *Micromelus subapterus* Riley. The seasonal history and field habits of these parasites will require another season's observation before they can be effectively treated. The scope of this paper is therefore limited to the life histories and relationships of these species to one another and to their common host as determined under laboratory conditions.

[1] The writer wishes to acknowledge his indebtedness to Messrs. E. O. G. Kelly, W. R. Walton, A. B. Gahan, W. R. McConnell, and J. A. Hyslop, all of the Bureau of Entomology, for helpful advice; to Mr. Kelly for making the work possible, and to Mr. Gahan for determining all specimens.

Journal of Agricultural Research, Vol. VI, No. 10
Dept. of Agriculture, Washington, D. C. June 5, 1916
dv K—34

METHODS OF BREEDING AND REARING

The adult parasites used in all experiments were kept in modified forms of the Doten cage.[1] One form, used when it was desired to confine a number of parasites together, consisted of two large, straight-sided vials of the same diameter, the mouths of which snugly fitted into a paper tube 1 inch long. This paper tube was held in shape by a layer of adhesive plaster around the outside. The cage was prevented from rolling by sticking a square of heavy cardboard to one side of the connecting tube. A label was pasted to the upper side of the tube for identification. One vial was kept dry and clean, while water and honey were supplied in the other.

The other form of Doten cage, used chiefly for isolating pairs and individuals, was simply a small, straight-sided vial into the mouth of which was fitted the open end of a slightly smaller, straight-sided vial. A small label was pasted on the side of the larger vial for identification. Cages of this kind were prevented from rolling by keeping them in shallow boxes with corrugated pasteboard-lined bottoms. Food and water were placed in the smaller vial.

In both forms of cages the water and the honey used for food were placed separately in small droplets on the upper surface inside the food vial. The honey used was the extracted form diluted with an equal amount of water. It was necessary to exercise considerable care not to place too large a drop of honey in a cage, because of its tendency to run down on the inside of the vial and to entangle the insects. Fresh water and honey were placed in the cages daily, and at least once a week the food vials of the cages were carefully cleaned to remove dried or soured honey. Replenishing the food and water in the cages once a day seemed sufficient to supply the needs of the parasites. It was often found necessary to make up a fresh supply of the honey because of souring or molding, especially in hot weather. Sterilizing the fresh supply by placing the dropper bottle containing it in boiling water for a few minutes caused it to remain sweet and usable much longer.

BREEDING THE PARASITES

To determine all the life stages from egg to adult involved the processes of exposing Hessian-fly puparia to parasites, dissecting the parasite eggs from the host puparia, and rearing, in little glass-cell cages devised for the purpose, the resulting parasite larvæ on Hessian-fly larvæ which were also dissected from puparia. Hessian-fly puparia contained in sections of wheat stems were first exposed to the adult parasites by placing the stems in the vial cages containing the adults. The stems remained in the cage for a day, or until a parasite was seen to oviposit in a flaxseed, when they were removed and the puparia dissected. The

[1] Doten, S. B. Concerning the relation of food to reproductive activity and longevity in certain hymenopterous parasites. Nev. Agr. Expt. Sta. Tech. Bul. 78, 30 p., 10 pl. 1911.

eggs of the three species studied were always found between the inner surface of the puparium and the larva itself. They were transferred separately, each to an unparasitized Hessian-fly larva which had been previously dissected from its puparium and placed in a little glass-cell cage of the following description:

Flat glass plates 1 inch by 1¼ inches square were used, in which hollows about the size and shape of a Hessian-fly puparium were ground in one surface, one hollow per plate, this work being done with a small carborundum grinder. After a host larva and a parasite egg had been placed in a hollow, the cell was closed by covering it with an ordinary glass cover slip. The cover glass was held in place by two little dabs of honey on its underside. The cell was not sealed by a complete ring of the adhesive because of the desirability of diffusion of atmospheric moisture under the cover slip. Honey seemed to be the ideal adhesive for this purpose, since it had no odor harmful to the inmates of the cell; it held the cover-glass tight against the slide; it did not dry so hard as to prevent the cover-glass from being easily removed; and a supply of it was always convenient. A label was pasted on the glass plate near one end for identification. The complete development of the parasite from egg to adult on its host could then be observed under the binocular in this little cell without disturbing the parasite or the host in the least.

With each of the three species the period from oviposition to emergence of adult, when individuals were reared in glass cells, approximated very closely the period from egg to adult when individuals were reared under the same meteorological conditions in Hessian-fly flaxseeds. Hence, the length of each stage of development as determined from individuals reared in glass cells may be considered normal.

It was discovered that the larvæ of all three species molted while making their growth within the little cells. The length of the instars was not observed, but the number of molts was determined by transferring to a balsam mount on a microscope slide all the material left behind in the little glass cell where a single individual had made its growth. In cases where the larva had pupated, the last molted skin was added to the mount. In cases where the full-grown larva had not pupated, the mandibles borne by the larva were dissected from it and added to the mount. To determine the number of molts of a single individual, the mount of the material it left behind was examined under the microscope and the number of pairs of mandibles in the material ascertained. In all cases the cell in which the larva made its growth was known to be absolutely clean when the host and the egg from which the parasite larva hatched were placed in it; hence, it was known that all pairs of mandibles found in a mount belonged to the same larva. Cells which contained simultaneously the remains of more than one parasite larva were not used in determining the number of molts. No attempt was made to determine the number of molts of individuals which had made their growth inside flaxseeds.

EUPELMUS ALLYNII

THE EGG

The egg of *E. allynii* French (Pl. LI, fig. 1) is elliptical in shape, with a thin stalk of varying length on one or both ends. In some cases the stalk seems to be entirely absent from one end. The egg is grayish white in color. The long axis of the body of the egg averages 0.35 mm., the short axis 0.14 mm. in length. As a parasite of the Hessian fly, the observations at Wellington, Kans., indicate that the egg is normally deposited in the puparium of the host. Females were repeatedly observed by Mr. E. O. G. Kelly and the author to be very numerous in fields, ovipositing in Hessian-fly flaxseeds where these constituted the only stage of the fly to be found. In one instance, however, a wheat stem containing nearly grown Hessian-fly larvæ, but no flaxseeds, was placed in a vial cage containing females of *E. allynii*. Upon dissecting this stem two eggs of this parasite were found inside the leaf sheath close beside the Hessian-fly larvæ. Whether or not the parasite is able to complete its development on Hessian-fly larvæ before they have formed puparia is still unsettled.

Hundreds of flaxseeds in which *E. allynii* had oviposited have been dissected and the eggs of the parasite have always been found inside the puparium but external to the inclosed Hessian-fly larva or pupa. Sometimes they were unattached, but more often the egg was fastened to the inner surface of the puparium by a little netlike structure made apparently of fine, white threads tangled together (Pl. LI, fig. 2). The threads forming the net appeared to be identical in diameter, color, and material with the egg stalks. The edges of this little net or mat were fastened down all around the egg, holding it securely in place. Sometimes the net was partly fastened to the host larva in addition to the puparium. In all experiments *E. allynii* oviposited seemingly indiscriminately in flaxseeds already containing parasite larvæ as well as in those containing Hessian-fly larvæ. The incubation periods of 109 eggs varied from 1½ days to 5 days. The egg stage was shorter in summer temperatures, observations being made during a period from July to November.

THE LARVA

Upon becoming fully formed inside the egg the larva (Pl. LII, fig. 2) breaks through one end of the chorion and after crawling around a little attaches itself to the external surface of the host larva. The parasite larva bears strong mandibles and feeds externally on the Hessian fly by puncturing the epidermis of the host and sucking out the body liquids. Larvæ reared in glass cells became full grown in from 7 to 10 days. After becoming full grown many of the larvæ were inactive for months; others pupated at once. In the warm summer temperatures most of the larvæ reared pupated at once upon completing their growth, while larvæ reared in the fall pupated only in occasional instances.

The larvæ reared in glass cells normally pass through five instars. Nearly all mounts made of the material left behind by larvæ which had finished feeding showed a total of five pairs of larval mandibles, while in the remaining mounts from two to four pairs were found which always correspond in size and shape to some one pair in the complete series. Five was the maximum number found in any one instance, and in cases where less than five were present it appeared that some of the molts had been lost in manipulation. Where five pairs of mandibles were found in a single mount, the sizes increased fairly uniformly from the second molt to the last. The mandibles and head shields of newly hatched larvæ appeared to be more heavily chitinized than those of later instars, except the last, and somewhat larger than those of the second instar. The mandibles of all instars are similar in shape. They articulate laterally with the head and fold together across the mouth, the ends overlapping. They are decidedly curved, taper to points, and are brown and chitinous. The sharp distal portions of the mandibles enlarge suddenly into a comparatively broad base bearing a chitinous lobe on the ventral side. The following average measurements will show the relative sizes of molted mandibles. These measurements represent the distance in a straight line, from the tip of the mandible to the shoulder, where the mandible suddenly enlarges into the broad basal portion.

Molt No.	Length of mandible.
1	0.016 mm.
2	.016 mm.
3	.024 mm.
4	.032 mm.
5	.048 mm.

The full-grown larva is grayish white, averaging about 3 mm. long and 0.9 mm. in diameter, with 13 body segments besides the head. There are no tubercles on the head, but there is a row of four hairs evenly spaced across the top. The front of the head bears a pair of hairs, one on each side, just outside of each of which is a very short, white, conical projection, apparently antennæ. There is a short bristle near the base of each mandible. The mouth is chitinized along its upper edge, this brown, chitinous rim extending around the bases of the mandibles and bearing six toothlike lobes pointing downward along the portion of the edge between the mandibles (Pl. LII, fig. 1). A subdorsal and sublateral row of fine, white hairs runs the full length of the body on each side, one hair per segment in each row. The first three body segments bear several additional rows. What appears to be the anal segment is divided into a dorsal and a ventral lobe by a transverse invagination across the end. The dorsal lobe bears two pairs of short, fine hairs, one pair close together near each lateral end of the lobe. The ventral lobe bears a short hair at each lateral end. The body hairs are evidently tactile organs, since when any of them are touched, the larva wriggles and bites viciously at the point of contact.

Larvæ of this species seem to be better equipped, more vigorous, and more capable of defending themselves than the larvæ of *Micromelus subapterus* and *Merisus destructor*. *E. allynii* was reared from egg to adult on larvæ of both the other species just mentioned as well as on the Hessian fly. In one case, however, a newly hatched larva of *E. allynii* placed on a full-grown larva of *M. subapterus* in a glass cell was killed by the latter almost immediately. A few instances were observed where larvæ of *E. allynii* killed other individuals of the same species present in the same Hessian-fly puparium.

THE PUPA

The larva forms a naked pupa (Pl. LI, fig. 3, 4) inside the puparium of the host. The first step in the process is the excretion of all waste matter from the body, leaving the larva pure white. The pupa is then formed and the last larval skin cast off. The newly formed pupa is nearly white, but turns dark within a few hours. The pupal stage of 30 specimens reared in glass cells varied from 9 to 24 days. The pupal period of those pupating in the summer averaged 13 days, while the pupal periods of those reared late in the fall became as long as 24 days in some cases. The arrival of cold weather retards pupal development, but whether or not the pupæ are able to survive severe winter temperatures has still to be determined. When the adult has completely developed, the pupal skin is cast off inside the host puparium, and the adult gnaws a round hole through the flaxseed near one end, penetrating the leaf sheath covering the flaxseed, through which it emerges.

THE ADULT

After remaining quiet until dry, the adult becomes very active. Adults do not seem to fly more than a few feet at a time, using their wings merely to go from stem to stem. They do this so quickly and often that it is difficult to observe a single individual in the field very long. The females run quickly up and down the wheat stems, vibrating their antennæ rapidly against the side of the stem until they come to a place where a Hessian-fly puparium is located. Here they feel back and forth above the flaxseed until they locate the exact spot which suits them for oviposition. Then, facing upward, the tip of the abdomen is bent down until it touches the stem and raised away again, leaving the ovipositor pressed vertically against the stem supported from its articulation with the middle ventral portion of the abdomen. The leaf sheath and puparium are pierced by what under the microscope appears to be a sort of drilling motion of the ovipositor, which seems to be rotated part way around and back again. Oviposition takes several minutes.

Males placed in the same cage with females usually attempt to mate with them at once. Mr. W. R. McConnell has ascertained that this species can reproduce parthenogenetically. The question of the sex

of parthenogenetic progeny has not yet been definitely settled. Mated females produced both male and female progeny. Two mated adults kept separately in vial cages from the time they emerged from pupæ until they died each laid a total of 58 eggs. This number actually was found in each case by dissection of flaxseeds which had been exposed to the adult. A few eggs may have been lost in dissection. These adults remained alive for periods of 48 and 56 days and were ovipositing during periods of 29 and 46 days, respectively. Another adult, caught in the field while ovipositing in a flaxseed, remained alive in a vial cage and oviposited in flaxseeds during a period of 57 days. An unmated female was kept alive in a vial cage for 83 days. How long adults normally live in the field is not known.

In one experiment Mr. W. H. Larrimer, of the Bureau of Entomology, exposed stems of *Elymus canadensis* containing galls of *Isosoma* sp. to two *Eupelmus allynii* females which previously had been ovipositing in Hessian-fly puparia. They at once oviposited in the galls. The galls were dissected and the inclosed larvæ of *Isosoma* sp., together with the eggs of *E. allynii* found in the galls, were transferred to glass-cell cages, one larva of *Isosoma* sp. and one parasite egg to each cell. The parasites proceeded to complete their development to adults on the larvæ of *Isosoma* sp. Progeny were also bred on the Hessian fly from the same parents used by Mr. Larrimer. These parents and their progeny were all determined by Mr. Gahan as *E. allynii*.

MERISUS DESTRUCTOR

THE EGG

The egg of *Merisus destructor* Say (Pl. LI, fig. 5) is elongate, kidney-shaped, circular in cross section, with one end smaller than the other. It is white, with the surface apparently smooth. The average length of eggs measured was 0.4 mm., the average diameter at thickest point, 0.1 mm. Hundreds of the eggs were dissected from flaxseeds, in which they had been deposited, and in all cases they were found external to the host larva or pupa inside the puparium. Some eggs apparently bore a short pedicel on one end, which seemed to be fastened to the inside of the host puparium. Ordinarily, however, the eggs were found free.

M. destructor, like *E. allynii*, normally oviposits in the Hessian-fly flaxseed, according to the observations of Mr. Kelly and the author at Wellington, Kans. It was very abundant in the fields at times when no other stage of the Hessian fly was present. The females were repeatedly observed ovipositing in puparia in the field. In cages they also oviposited readily in flaxseeds contained in sections of wheat stems as well as in naked flaxseeds removed from stems. They did not oviposit readily in sections of stems containing only partially grown Hessian-fly larvæ, although they seemed interested in them. In one instance,

however, a female *M. destructor* oviposited in a stem containing nothing but partially grown larvæ. Upon dissection the egg was found sticking to the stem underneath the leaf sheath, close to one of the larvæ. It is not yet known whether or not *M. destructor* can develop to maturity on partially grown Hessian-fly larvæ. The egg stages of 96 specimens placed on Hessian-fly larvæ in glass slides varied from 1½ days in hot July weather to 4 days in cool September weather. The larva emerges from the egg by breaking through one end. After crawling around a little the larvæ reared in glass cells fastened themselves with their mandibles to the outside of the host larvæ in order to feed.

THE LARVA

The full-grown larva of *M. destructor* (Pl. LII, fig. 4) is white with the dingy brown contents of the alimentary tract visible through the integuments. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair and each bears a small conical projection, evidently an antenna, varying from white to pale brown in color and about 0.02 mm. long. The median ventral surface of the head bears the round suctorial mouth opening. The only mouth appendages distinguishable are a pair of brown chitinous mandibles borne laterally and closing together across the mouth with their tips overlapping (Pl. LII, fig. 3). The distal portion of the mandible is conical, tapering gradually to a sharp point. The proximal end is suddenly enlarged, evidently to provide for muscle fastenings. One subdorsal and one sublateral row of very short and inconspicuous setæ on each side of the body are clearly distinguishable in some specimens, extending the full length of the body, one seta per segment in each row. On some specimens there appear to be two ventral and two dorsal rows of scarcely discernible setæ on the first three body segments only. There are thirteen body segments besides the head, the anal segment being divided into a dorsal and a ventral lobe by a horizontal fold across the end. The dorsal lobe bears four very short, fine setæ in a row across the end, the setæ composing the row being usually in two lateral pairs. The ventral anal lobe bears only two setæ, one near each lateral end of the lobe. The length of the full-grown larvæ averages 2.5 mm., the largest diameter, 0.7 mm.

Balsam mounts of all the material left behind in the little glass cells by pupating larvæ nearly always contained five pairs of mandibles. Mounts of all the material left in the cell by full-grown larvæ which had ceased to feed, together with the mandibles dissected from such larvæ, also nearly always contained five pairs of mandibles. In every mount the pairs varied uniformly in size from those resembling the ones borne by newly hatched larvæ to those borne by full grown larvæ. Mandibles of newly hatched larvæ were somewhat hooked. All the remaining pairs were similar in shape, and corresponding pairs in all the mounts

were almost identical in size. As was the case with *E. allynii*, the mandibles of the newly hatched larva appeared to be heavier, more powerful, and somewhat larger than the mandibles borne by the second-instar larva. Also, the head shield appeared to be more heavily chitinized in the first instar than in the later ones. Beginning with the second instar, the successive pairs of mandibles apparently increase fairly uniformly in size with each molt. In the mounts where five pairs of mandibles could not be found, those which were found correspond in size and shape to some one of the pairs in the complete series and it was evident that certain pairs had been lost in making the mount. No more than five pairs were found in any one case. All the findings lead to the conclusion that larvæ of *M. destructor* normally pass through five instars in making their growth.

The relative sizes of the molted mandibles are shown below. The measurements represent the distance in a straight line from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

Molt No.	Length of mandible.
1	0.014 mm.
2	.014 mm.
3	.020 mm.
4	.024 mm.
5	.032 mm.

The larvæ develop readily on Hessian-fly larvæ and pupæ, both in flaxseeds and in glass cells, unless the host pupa has nearly completed its development. Several newly hatched larvæ in flaxseeds and glass cells containing Hessian-fly pupæ which were nearly developed killed the pupæ, but died from lack of sufficient food to complete their growth. The larvæ are evidently cannibalistic upon occasion. In one flaxseed which had been exposed to ovipositing females, a young larva of *M. destructor* was found which had been feeding, as also the shrunken remains of another young larva. Evidently the healthy larva had found and killed the other and was feeding on the Hessian-fly larva when the flaxseed was dissected. Full-grown larvæ in glass cells punctured and killed eggs and larvæ of *M. destructor* which were placed in the cells with them. Larvæ of *M. destructor* were able to become full grown by feeding on larvæ of *M. subapterus* also.

The periods required by 36 larvæ to make their growth when reared in glass cells varied from 7 to 11 days. Cool weather appeared to make growth slower. After becoming full-grown the majority of the larvæ of *M. destructor* reared in glass cells remained quiescent for months, though still alive and able to wriggle vigorously when touched. Larvæ reared in flaxseeds exhibited the same characteristic. In other words, the larvæ seem to have a tendency to estivate and hibernate until another warm season before pupating. Larvæ of *M. destructor* were actually found to

have hibernated in stubble of wheat cut the previous June. Eight per cent of the flaxseeds in stubble gathered from a field in southeastern Kansas in late March contained live, full-grown parasite larvæ which afterwards became adult and were determined by Mr. Gahan as *M. destructor*.

THE PUPA

The period from the formation of the pupa (Pl. LI, fig. 6) to the emergence of the adult varied from 7 to 14 days in 21 specimens carried through this stage in glass cells. Those pupating in April and September, when cooler temperatures prevailed, took longer to develop than those which pupated during the hot weather of July and August. The larvæ form naked pupæ inside the puparium of the host. The process of pupation as observed in glass cells begins with the excretion of all waste matter from the body of the larva, which then becomes pure white. In a few hours the pupa is formed. The eyes begin to turn reddish in about a day and by the fourth day are a very dark red. The body of the pupa is by the fourth day a creamy white, and by the sixth day the head and thorax are black. Within another day the abdomen turns black except for the base of the abdomen, which assumes the light brown as found in males and some females. The emergence of the adult follows within a day or so after the pupa has turned dark. Cool weather retards development. The adult casts off the pupal skin inside the host puparium and emerges by gnawing a round hole through the side of the flaxseed and the wheat leaf sheath covering it just large enough for the adult parasite to crawl through.

THE ADULT

Adults soon become active after emerging from flaxseeds. In the spring males emerged two or three days before the females in cages containing stubble collected from the fields where it had stood during the winter. Mating took place at once when the females emerged. Oviposition takes place in the following manner: The females run up and down the wheat stalks, vibrating their antennæ rapidly against the side of the stem. When they come to a place where there is a flaxseed underneath the leaf sheath, they stop and excitedly feel up and down over the place where the flaxseed is located. They face upward to oviposit, with the body parallel to the puparium. They locate the proper place for oviposition with the tip of the abdomen and then raise it away from the stem, leaving the ovipositor unsheathed and pointing perpendicularly against the stem from its articulation with the middle of the abdomen. In less than a minute the ovipositor is forced through the leaf sheath and the puparium. In penetrating the flaxseed the ovipositor is seemingly rotated like a drill part way round and back again. Oviposition takes 5 to 10 minutes, and dissections of flaxseeds indicate that a single egg is laid at a time. One female kept isolated in a vial cage laid a total of 39 eggs in puparia exposed

to her and later dissected. Some may have been lost in dissection. This female was laying eggs during a period of six weeks. Other females were kept alive and active in confinement for periods of over two months.

Some stems of *Elymus canadensis* containing galls formed by a species of Isosoma were placed in a vial cage containing females of *M. destructor*. Almost immediately one of the females became interested in the galls, feeling over them with her antennæ. She then attempted to oviposit, endeavoring persistently to penetrate the gall with her ovipositor, but without success. Mr. W. H. Larrimer finally succeeded in getting the females to oviposit in the Isosoma galls and found the eggs inside the galls but external to the larvæ of *Isosoma* sp. He actually reared a few specimens of *M. destructor* from egg to adult on the Isosoma larvæ in glass cells. The parents used in this experiment and the progeny which were reared were determined as *Merisus destructor* by Mr. Gahan.

MICROMELUS SUBAPTERUS[1]

Heretofore it has been uncertain that the winged and wingless forms of *Micromelus subapterus* Riley were the same species. It has been proved, however, that the two forms are specifically identical by breeding a wingless female from a winged parent. Further evidence indicating that the winged and wingless forms are the same species is the fact that wingless males mated with winged females as readily as with the wingless form. The method by which the wingless female was bred from the winged parent is as follows: The winged parent deposited an egg in a Hessian-fly puparium known to have been previously unparasitized. The egg was removed from the puparium and from it a wingless adult was reared on a healthy Hessian-fly larva, which also had been dissected from its puparium. Mr. Gahan found this wingless offspring of a winged adult to be identical with winged specimens of unknown parentage.

THE EGG

The egg of *Micromelus subapterus* (Pl. LI, fig. 7) resembles that of *Merisus destructor* in size and shape. It is elongate, kidney-shaped, with one end longer than the other, circular in cross section, white in color, with surface of shell smooth, and about 0.38 mm. long by 0.09 mm. in diameter at the thickest point. It has no stalk.

All the observations made at the Wellington (Kans.) station lead to the conclusion that the egg is normally laid in the Hessian-fly puparium. In cages the adults oviposit readily in flaxseeds, the eggs being placed inside the puparia but external to the inclosed Hessian-fly larvæ and unattached. This was the case both when stems of fly-infested wheat

[1] Mr. A. B. Gahan makes the following statement: "The real generic position of this species is in doubt. It was originally described by Riley under the name *Merisus* (*Homoporus*) *subapterus* Riley, and later referred to Boeotomus by Osborn and other writers. N. V. Kurdiumov has more recently placed the species in the genus Micromelus. Doctor Ashmead reduced Boeotomus to synonymy with *Micromelus*."

were exposed to the parasite in vial cages and when ovipositing females were placed in large glass chimneys containing growing wheat infested with the Hessian fly. Occasionally the females have been observed apparently to oviposit in stems containing only larvæ; and although careful dissections of these stems were made, no eggs were found. Further proof that *M. subapterus* normally oviposits in flaxseeds was obtained by dissecting puparia collected in fields where this parasite was numerous at the time the collection was made. Both eggs and young larvæ of a parasite were present in the flaxseeds and when reared to maturity in the laboratory were found to be *M. subapterus*. The egg stage in 119 cases varied from 1½ to 5 days. Low temperatures in fall and spring retarded embryonic development. The larvæ reared in glass cells emerged from the eggshells by breaking through one end, and after crawling around a short time settled down in one place to feed.

THE LARVA

The full-grown larva of *M. subapterus* (Pl. II, fig. 6) averages 2 mm. long by 0.75 mm. in thickness. It is white, with the pale-brown contents of the alimentary tract showing through the body. There are two pairs of slightly raised circular tubercles on the front of the head near the top. The lower pair are slightly farther apart than the upper pair, the former each bearing a small conical projection, evidently the antennæ, varying from white to brown and about 0.015 mm. long. The median ventral surface of the head bears the round, suctorial mouth opening. The only mouth appendages distinguishable are a pair of very small brown chitinous mandibles borne laterally and closing together across the mouth (Pl. LII, fig. 5). The distal ends of the mandibles are sharp and needlelike. The proximal ends are suddenly enlarged, evidently to provide for muscle fastenings. A minute pit, which sometimes appears to have a brown center, occurs on each side of the mouth. The body is entirely glabrous, so far as could be determined, except for the anal segment, oval in shape, with the anal end the more pointed. There are 13 segments besides the head, the anal segment being divided into a dorsal and ventral lobe by a horizontal fold across the end. The dorsal lobe bears four short, very fine setæ in a transverse row, these usually being in lateral pairs. The ventral anal lobe bears only two very short, fine setæ, one near each lateral end of the lobe.

The number of instars passed through by larvæ of *M. subapterus* in making their growth appeared to be five. Five pairs of molted mandibles increasing uniformly in size, from the small pair resembling those borne by newly hatched larvæ to the large pair molted off when full-grown larvæ pupated, were present in almost every mount made of the material left behind in a cell where a larva had developed. In

mounts where five pairs could not be found, each of those present corresponded to some one of the pairs in the complete series. No more than five pairs were found in a single mount. As in the two species of parasites previously discussed, the head shield of the newly hatched larva was more heavily chitinized than those of later instars. The mandibles appeared to be more powerful for their size than those of any later instar, and in some cases they were actually larger than the second-instar mandibles. The approximate sizes of the respective pairs of molted mandibles follow. The measurements represent the distance from the tip of the mandible to the shoulder where it suddenly enlarges into the broad base.

Molt No.	Length of mandible.
1	0.012 mm.
2	.012 mm.
3	.016 mm.
4	.020 mm.
5	.028 mm.

Larvæ of *Micromelus subapterus* do not seem as capable of moving around and reattaching themselves to the host as are the larvæ of *Eupelmus allynii* and *Merisus destructor*. Larvæ reared in glass cells crawled about a little immediately after hatching before they settled down to feed, but they usually completed a large part of their growth without leaving the original feeding point on the external surface of the host.

This species not only developed on Hessian-fly larvæ in puparia, but in some instances fed on the larvæ of other parasites. One egg of *Micromelus subapterus* was placed on a full-grown larva of the same species in a glass cell. The egg hatched and the little larva became full grown on the large larva, almost completely devouring it. Another egg of *M. subapterus* was placed on a full-grown larva of *Merisus destructor* and the little larva hatching from the egg became full grown on the larva of *Merisus destructor*. Experiments like these, however, usually resulted in the destruction of the egg or young larva of *M. subapterus* and the survival of the full-grown larva of the same or the other species as the case happened to be. Larvæ of *M. subapterus* apparently could make their growth on the Hessian-fly pupa as well as on the larva unless the former had partially developed. Where the host pupa had already completed a large part of its development, both the host and the parasite generally died, the latter apparently for lack of sufficient suitable food. Larvæ of *M. subapterus* appeared to be the least able to defend themselves where the larvæ of more than one species occurred in the same flaxseed. They also seemed the least capable of successfully establishing a feeding point on the host larva, at least when reared in little glass cells. They seemed more delicate in structure and less vigorous.

The respective periods required for 36 larvæ to make their growth varied from 7 to 10 days. A large proportion of the larvæ after finishing

their growth remained in a quiescent state in the little glass cells for months. Others pupated at once upon completing their growth.

THE PUPA

In general, the process of pupation as observed in glass cells is as follows: The full-grown larva excretes all waste matter from the body, leaving it perfectly white. Within a day after this operation the pupa (Pl. LI, fig. 8) is formed and is at first perfectly white, the last larval skin being found at the anal end of the pupa. In another day or so the pupa begins to turn a pale brown, and the eyes turn reddish. The pupa finally becomes entirely black as development progresses, the head and thorax changing first, and remains so until the adult emerges.

The pupa is formed naked inside the puparium of the host. The adult emerges by casting off the pupal skin inside the host puparium and then cutting a round hole through the side of the flaxseed near one end. The length of the pupal period varied in 21 instances from 7 to 13 days. Cool weather retarded the development of the pupæ. A larger proportion of the larvæ reared in the cooler weather of fall pupated at once upon attaining their growth than was the case with the larvæ reared in the hot weather of midsummer, indicating a tendency of the larvæ of this species to estivate.

THE ADULT

Newly emerged adults became active almost at once upon emerging from the host puparium. Males placed in the same cage with females began mating at once. Females that had been mated seemed to oviposit more readily than unmated females. Both Mr. McConnell and the writer found that this species was arrhenotokous in every instance where this point was determined. Females have been kept alive in cages as long as six months, and one female oviposited after having been kept alive over five months. It was usual for them to live and oviposit for at least a month in vial cages. One female actively oviposited during a period of 75 days and laid a total of 103 eggs. Another female laid a total of 45 eggs. The number of eggs laid by a single female was determined by exposing flaxseeds to an isolated individual and dissecting them to find the number of eggs the parasite had laid in each.

In ovipositing the female would run up and down the stems of the plants, vibrating her antennæ against the surface. When she came to a place in the stem where a flaxseed was located, she would stop, feel up and down over the spot with her antennæ, and then lower the tip of her abdomen. When she had found the point that suited her for oviposition, the end of the abdomen was raised, leaving the ovipositor standing vertically against the side of the stem from its articulation with the middle of the abdomen. In penetrating the leaf sheath and puparium the parasite seemed to rotate the ovipositor with a drilling motion in

addition to the downward pressure exerted on it. The female always took a position heading up the stem in ovipositing. The whole process generally took five minutes or more.

CONCLUSION

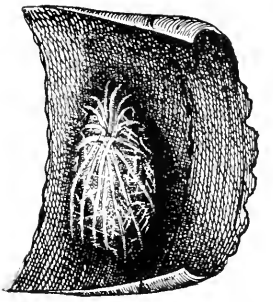
The writer's experiments and observations have all led to the inference that only one specimen of any of the three species studied ever matures in a single Hessian-fly puparium. In every instance where more than one egg or larva was placed on the same host or in the same cell, one survived and the rest were killed by that one, or starved to death. This was true whether the two or more larvæ were of the same or different species.[1]

[1] For correct figures of the adults of all three of the species treated in this paper, see U. S. Dept. Agr. Farmers' Bul. 640. (Webster, F. M. The Hessian fly. 29 p., 17 fig. 1915.)

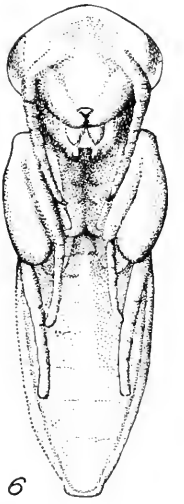
PLATE LI

Fig. 1.—Egg of *Eupelmus allynii*.
Fig. 2.—Egg of *Eupelmus allynii* in situ.
Fig. 3, 4.—Pupa of *Eupelmus allynii*.
Fig. 5.—Egg of *Merisus destructor*.
Fig. 6.—Pupa of *Merisus destructor*.
Fig. 7.—Egg of *Micromelus subapterus*.
Fig. 8.—Pupa of *Micromelus subapterus*.

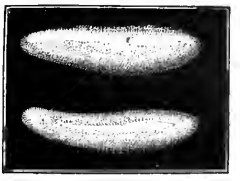
8



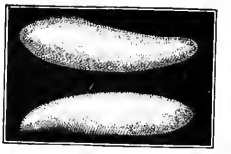
2



6



7

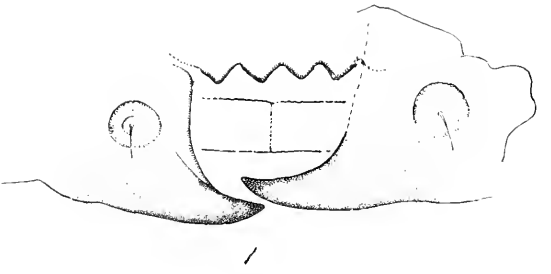


5

3

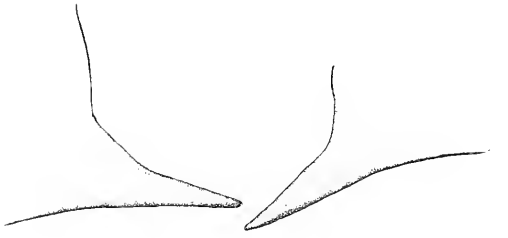
1

4

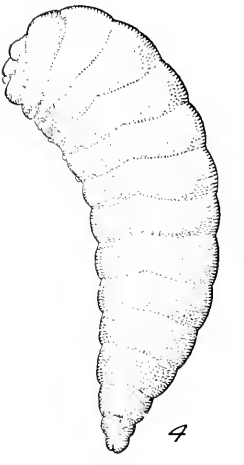


1

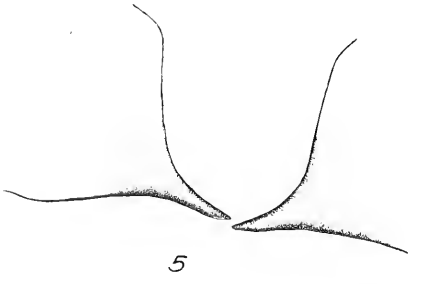
2



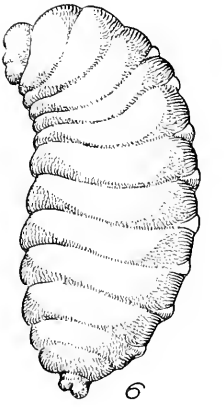
3



4



5



6

PLATE LII

Fig. 1.—Mandibles of full-grown larva of *Eupelmus allynii*.
Fig. 2.—Larva of *Eupelmus allynii*.
Fig. 3.—Mandibles of full-grown larva of *Merisus destructor*.
Fig. 4.—Larva of *Merisus destructor*.
Fig. 5.—Mandibles of full-grown larva of *Micromelus subapterus*.
Fig. 6.—Larva of *Micromelus subapterus*.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
20 CENTS PER COPY
SUBSCRIPTION PRICE, $3 PER YEAR

Vol. VI JUNE 12, 1916 No. 11

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., JUNE 12, 1916 NO. 11

EFFECT OF RÖNTGEN RAYS ON THE TOBACCO, OR CIGARETTE, BEETLE AND THE RESULTS OF EXPERIMENTS WITH A NEW FORM OF RÖNTGEN TUBE

By G. A. RUNNER,
Entomological Assistant, Southern Field Crop Insect Investigations, Bureau of Entomology

INTRODUCTION

The Röntgen tube used in experiments on the effect of Röntgen rays on the tobacco, or cigarette, beetle (*Lasioderma serricorne* Fabricius) described in this paper is a new form designed by Coolidge.[1] By this type of tube a much more powerful Röntgen-ray radiation can be maintained than was possible with the apparatus used in experiments of a similar nature previously made by the writer. The intensity and the penetrating power of the Röntgen rays produced are both under the complete control of the operator, and many of the factors limiting the use of other types of tubes for the special purpose desired are absent. The tube can be operated continuously for long periods without showing an appreciable change in either the intensity or the penetrating power of its resulting radiation. The starting and running voltage are the same. The resulting radiation is therefore homogeneous and of any desired penetrating power.

The ordinary forms of tubes used in previous experiments were incapable of being operated continuously without change in penetrating power. Owing to the fluctuation in intensity and penetrating power incidental to frequent adjustment, it was impossible to tell with any degree of accuracy the dosage and amount of radiation.

In previous experimental work with Röntgen rays it had been found that in sterilizing cigars or tobacco, small dosages are ineffective, from a practical standpoint. To be effective, the radiation must be intense, and it is evident that if the process can be successfully applied to commercial work, the apparatus used must be capable of producing and maintaining such radiation during the entire period required for the material treated to pass through the exposure chamber of the machine.

[1] Coolidge, W. D. A powerful Röntgen ray tube with a pure electron discharge. *In* Phys. Rev., s. 2. v. 2, no. 6, p. 409–430, 6 fig. 1913.

TABLE I.—*Effect of Röntgen rays on development of the tobacco beetle*

[Experiments made at Schenectady, N. Y.[1]]

Experiment No.	Date.	Stage of insect.	Number tested.	Age.	Current (milliampere minutes).	Time.	Exposure (milliampere minutes).	Date examined.	Results.	Date examined.	Results.
				Days.		*Minutes.*					
1	Apr. 10	Egg.....	27	1	10	15	150	Apr. 16	None hatched.....	May 1	None hatched.
2	Apr. 12	...do.....	95	1–2	15	10	150	Apr. 20	.....do.....	...do.....	Do.
3	Apr. 13	...do.....	35	1	15	10	150	May 1	.....do.....	Sept. 11	Tobacco uninjured and uninfested. Check heavily infested.
4	Apr. 14	...do.....	50	1	15	10	150	...do.....	.....do.....	...do.....	Do.
5	Apr. 15	...do.....	50	1	15	10	150	...do.....	.....do.....	...do.....	Do.
6	...do.....	...do.....	50	3	15	10	150	...do.....	.....do.....	...do.....	Do.
7	Apr. 16	...do.....	50	2	15	10	150	...do.....	.....do.....	...do.....	Do.
8	...do.....	...do.....	110	1	15	10	150	...do.....	.....do.....	...do.....	Do.
9	...do.....	...do.....	50	2	15	10	150	...do.....	.....do.....	...do.....	Do.
10	...do.....	...do.....	50	2	15	10	150	...do.....	.....do.....	...do.....	Do.
11	Apr. 17	...do.....	50	3	15	10	150	...do.....	1 eggshell and 1 live larva.....	...do.....	1 dead larva partly grown. Check infested. Larvæ, pupæ, and adults found.
12	...do.....	...do.....	50	3	15	10	150	...do.....	None hatched.....	...do.....	No signs of development. Tobacco uninjured. Check infested.
13	...do.....	...do.....	42	4	15	40	600	...do.....	.....do.....	...do.....	Do.
14	...do.....	...do.....	52	5	15	40	600	...do.....	38 eggs hatched; 14 sterile.....	...do.....	Several small dead larvæ found; no pupæ or adults; no evidence of any of the larvæ having developed.
15	...do.....	...do.....	52	1	15	10	150	...do.....	None hatched.....	...do.....	Tobacco uninfested and uninjured. Check box infested.
16	June 7	...do.....	203	1–2	15	10	150	June 24	.....do.....	Sept. 1	Do.
								July 11	.....do.....	Dec. 12	No evidence of any of the eggs having hatched.
17	...do.....	...do.....	20	1–3	15	10	150	June 24	.....do.....	Sept. 1	Do.
								July 11	.....do.....	Dec. 12	Do.
18	...do.....	...do.....	82	4–5	15	10	150	June 24	2 dead larvæ in first instar; a few eggshells; no live larvæ.	Sept. 1 Dec. 12	No signs of further development. Tobacco uninfested.
19	Apr. 17	Larval..	21	(2)	15	40	600	May 3	All alive.....	.....	
								May 28	14 alive; 7 dead.....	.....	
								July 11	2 alive; rest dead.....	.....	
								Sept. 1	All dead; none reached adult stage; all check larvæ transformed by July 11.	.....	

20	June 7	...do.....	35	(3)	15	40	600	June 24	21 alive; part seem inactive.....	Sept. 1	9 alive...	No signs of feeding observed.
										Oct. 1	5 alive...	None of the larvæ reached the pupal stage. Treatment apparently stopped all development and activity; were somewhat shrunken and of an abnormal whitish color.
										Nov. 1	4 alive...	
										Dec. 11	2 alive...	
								July 11	12 alive, but in a dormant condition; none have transformed to pupal or adult stage; checks have all transformed to adults.	Jan. 1, 1916.	2 alive...	
										Jan. 10, 1916.	All dead	
21	...do.....	Pupal...	20	.....	15	40	600	June 16	8 alive; none transformed.....	Aug. 1	Adults dead. No eggs laid.	
								July 11	4 live adults found.....			
22	Apr. 17	Adult...	67	.....	15	40	600	Apr. 17	Alive and active directly after exposure.	Oct. 1	Tobacco from jar containing exposed beetles and eggs examined; no signs of infestation.	
								Apr. 22	Large number of eggs deposited. 208 eggs kept under daily observation; rest of eggs placed with chewing tobacco in sealed jar.			
								May 30	None of the eggs hatched; eggs from check beetles hatched normally.			
23	June 7	...do.....	50	.....	15	40	600	June 24	26 alive, 24 dead; large number of eggs found; none hatched; check beetles, 32 alive, 18 dead; eggs from check beetles hatched normally.	July 24	All adults in both exposed and check lot dead; none of the eggs from exposed beetles hatched.	
								July 11	Exposed lot, 7 alive; check, 5 alive; 126 eggs kept from exposed lot examined; none hatched.	Sept. 1	No signs of infestation in tobacco kept in jar which contained exposed adults. Tobacco in check jar infested.	

[1] The following were constant in all the experiments: Material exposed 7.5 inches from focal spot of tube; spark gap 5.5 inches, giving, at a humidity of 57°, a voltage of about 65,000.

[2] Partly grown.

[3] Partly grown; some nearly mature.

EXPERIMENTAL WORK

Eggs for the experiments were obtained by placing large numbers of tobacco beetles in jars containing leaf tobacco which had been sterilized by heat. The eggs were then placed between slabs of chewing tobacco in wooden boxes. The covers of the boxes were tightly sealed with adhesive tape. Control boxes containing approximately the same number of eggs as the treated boxes were prepared in a similar manner.

Infested tobacco containing larvæ, pupæ, and adults was also exposed in sealed wooden boxes. After exposure the insects were transferred to wooden boxes containing granulated tobacco which had been sterilized by heat. A corresponding number of specimens were kept as controls.

Exposure to the rays was made by placing the containers directly under the Röntgen tube at a distance of 7.5 inches from its focal spot. In order to guard against any effect of heat, a fan was kept blowing on the container while the exposure was made. The maximum temperature registered by a thermometer placed in the chamber was 91° F.

In the series of experiments tabulated 150 milliampere minutes (current of 15 milliamperes for 10 minutes or a current of 10 milliamperes for 15 minutes), with a voltage of 65,000, was the minimum dosage applied.

The material used in the experiments was kept under observation until January 10, 1916. Table I gives the details of the experiments. The notes included show the condition at different times. During the colder months the material was kept in an automatically regulated electric incubator in which suitable breeding conditions were maintained. The temperature was kept at 86° F. and the humidity at 80.

Eggs from exposed beetles were kept under daily observation. Part were kept in cells on microscope slides and part were kept on the leaf tobacco on which they were laid and placed between slabs of chewing tobacco. Most of the eggs which failed to hatch became shrunken and changed in color in about 10 days. Part remained plump and apparently normal for a considerable time. In eggs which were over 2 days old and in which embryonic development was well advanced when treated the partly developed larvæ could be seen within by examination with a microscope.

As will be seen in Table I (experiments 11, 14, and 18), hatching took place in some of the eggs which were over 3 days old. In experiment 14, which was made with eggs nearly hatched when treated, part of the eggs hatched, even though the dosage of 150 milliampere minutes, which was effective with the newly laid eggs, had been increased to 600 milliampere minutes.

Results of previous experiments, as well as those tabulated, indicate that in treatment of the egg stage heavier dosages are required to sterilize eggs which are nearing the end of the incubation period than are required to sterilize eggs newly laid.

In these experiments the larvæ hatched from treated eggs failed to develop. In several other series of experiments with Röntgen rays made by the writer and also in experiments made by Morgan and the writer,[1] eggs given lighter dosage hatched and development seemed normal, several generations of tobacco beetles being reared from some of the tobacco and cigars which contained treated eggs.

In the two experiments with larvæ (No. 19 and 20), no immediate effect as the result of exposure to the rays was noted. After a time the larvæ became inactive, somewhat shrunken, and changed in color, and no evidence of feeding could be observed. Nearly all remained in an inactive or dormant condition for long periods before death. Two larvæ exposed on June 7 (experiment 20) remained alive until January 10, 1916. All check larvæ used in this experiment had transformed to the adult stage by July 11. All treated larvæ died before reaching the pupal stage. With conditions under which the material used in the experiments was kept, the normal larval period of the tobacco beetle is about 40 days. All larvæ used in the experiments were partly grown when the experiment was made. No further growth could be noticed. In general, the effect of the heavy exposure given (600 milliampere minutes, voltage 65,000, distance from focal spot of Röntgen tube 7.5 inches) seems to have been to stop development and activity and to produce an inactive or dormant condition, and greatly to prolong the larval period.

The results of all previous experiments with larvæ given comparatively light exposures had shown entirely negative results.

In the experiment with pupæ (experiment 21) the number of pupæ used was not sufficiently large to permit the drawing of positive conclusions. Of the 20 specimens treated, only 4 reached the adult stage. These seemed normal, but died without laying eggs.

In the two experiments with adults (experiments 22 and 23), the results obtained were very similar. The exposure given apparently did not affect the length of life or the activity. Mating was observed and large numbers of eggs were laid. None of the eggs from the exposed beetles hatched, while eggs from the check beetles hatched normally.

Egg clusters of the tent caterpillar (*Malacosoma americana* Fabricius) and the white-marked tussock moth (*Notolophus leucostigma* Smith and Abbot) were used. With both of these species the period of incubation is very long, eggs deposited in summer or fall not hatching until the following season. An exposure of 150 milliampere minutes was given. Other conditions of the experiment were the same as in experiment 7 made with eggs of the tobacco beetle, details of which are given in Table I. The experiment was made on April 16. The egg clusters treated contained something over 1,000 eggs of each species. The same number of clusters were kept as checks. Both experiments gave nega-

[1] Morgan, A. C., and Runner, G. A. Some experiments with Röntgen rays upon the cigarette beetle Lasioderma serricorne Fabr. *In* Jour. Econ. Ent., v. 6, no. 2, p. 226–230. 1913.

tive results, hatching being apparently normal in treated eggs of both species.

The eggs of both the tent caterpillar and the tussock moth were nearing the end of the incubation period when treated. In eggs of the tent caterpillar embryonic development is practically completed in the fall, the larvæ remaining in the eggshells over the winter and emerging on the appearance of warm weather in the spring.

SUMMARY

Under laboratory conditions tests made with a Röntgen-ray tube permitting a high-energy input and giving an intense and powerful radiation gave results which promise that the X-ray process may be successfully used in treatment of cigars or tobacco infested with the tobacco, or cigarette, beetle.

Heavy dosages must be given, as is indicated by the exposure given in the series of experiments tabulated in this paper.

In treatment of the egg stage, heavier exposures are required to sterilize eggs which are near the hatching point than are required to sterilize eggs newly laid.

In experiments performed by the writer a dosage equivalent to 150 milliampere minutes exposure with a spark gap of 5.5 inches gave satisfactory results with eggs in tobacco placed 7.5 inches from the focal spot of the tube. With this exposure the eggs in which embryonic development was well advanced hatched, but in all cases where these larvæ were kept under observation they failed to reach the adult stage.

The minimum lethal dosage at a given distance from the focal spot of the Röntgen tube used has not been determined.

In two separate experiments adults were given an exposure of 600 milliampere minutes (amperage × time), with a spark gap of 5.5 inches, giving an approximate voltage of 65,000, with humidity at 57. The distance from the focal spot of the Röntgen tube was 7.5 inches. The results are as follows:

(1) No effect on length of life was apparent, as the beetles died at about the same rate as the same number of beetles kept as a check.

(2) Large numbers of eggs were deposited after exposure. These eggs were infertile. Eggs laid by the check beetles hatched normally.

Larvæ were given an exposure of 600 milliampere minutes, other conditions of the experiment being the same as in the experiments with adults given above. While no immediate effect was apparent, the treatment had the effect of stopping activity and development, the larvæ remaining in a dormant condition for a prolonged period. All treated larvæ died before reaching the pupal stage.

STIMULATING INFLUENCE OF ARSENIC UPON THE NITROGEN-FIXING ORGANISMS OF THE SOIL

By J. E. GREAVES,
Bacteriologist, Utah Agricultural Experiment Station

INTRODUCTION

Arsenic, when applied to a soil, has been found to stimulate the ammonifying (Greaves, 1913c)[1] and especially the nitrifying organisms of that soil. The stimulation varied greatly with the form, quantity, and method of applying the arsenic. Furthermore it was found that very large quantities of arsenic had to be applied to a soil before its toxic effect became marked. This toxic effect became pronounced only when quantities of arsenic which far exceeded those found in any of the cultivated soils (Greaves, 1913b) had been applied. Therefore it was desirable to determine its influence and mode of action upon the nitrogen-fixing powers of the soil. For, even though arsenic does not inhibit the action of the ammonifiers or nitrifiers, if it stops or materially retards the nitrogen-fixing organism, it can not be said that arsenic is not injurious to the soil flora. To determine this point the following study has been made.

EXPERIMENTAL WORK

The soil used in the first part of this work was the same as that used by the author in the previous series. It is a typical bench soil, a sandy loam fairly high in calcium and iron content and supplied with an abundance of all the essential elements of plant food with the exception of nitrogen, which was low, a characteristic of arid soils.

The determination of the nitrogen-fixing powers of the soil was made as follows: Tumblers covered with Petri dishes were sterilized, and into these were weighed 100-gm. portions of the air-dried soil and 2 gm. of mannite, which were then carefully mixed. Sodium arsenate was added from a standard solution with the proper proportion of sterile distilled water and the mixture thoroughly stirred with a sterile spatula. The other arsenical compounds were added in the dry state and then carefully mixed. Sufficient sterile distilled water was added to make the moisture content of the soil 18 per cent. The tumblers and contents were weighed and the moisture content made up weekly to the initial concentration.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 414-416.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
dw

Vol. VI, No. 11
June 12, 1916
Utah—2

The samples were incubated at 28° to 30° C. for 18 days and the total nitrogen determined. The tumblers and contents at the end of this time were placed in an electric incubator and kept at 95° C. until dry. The soil was then ground in a mortar, after which 20-gm. portions were weighed and placed in Kjeldahl flasks. The nitrogen was then determined according to the Lipman and Sharp (1912) method. The determinations were all made in duplicate and compared with sterile blanks, so that each result reported is the average of two or more closely agreeing determinations. The compounds used were sodium arsenate, lead arsenate, cupric aceto-arsenite (Paris green), arsenic trisulphid, and zinc arsenite. In each case the quantity of the compound added was such as to give equivalent quantities of arsenic. The results reported as milligrams of nitrogen per 100 gm. of soil are given in Table I.

TABLE I.—*Quantity of nitrogen (milligrams) fixed in 100 gm. of soil during 18 days with varying amounts and different forms of arsenic*

Arsenic.	Sodium arsenate.	Lead arsenate.	Paris green.	Arsenic trisulphid.	Zinc arsenite.
P. p. m.					
0	18. 2	16. 1	15. 22	9. 8	9. 1
20	22. 4	16. 0	13. 72	11. 2	11. 9
40	14. 0	16. 4	13. 02	14. 0	9. 7
80	14. 0	18. 9	14. 00	15. 4	9. 6
120	15. 0	21. 0	8. 82	16. 2	10. 5
160	14. 4	21. 0	8. 32	16. 4	9. 7
200	14. 0	21. 7	7. 42	14. 0	8. 4
240	12. 6	16. 8	6. 72	12. 8	8. 4
280	0	16. 1	6. 02	11. 2	8. 4
320	0	16. 0	6. 00	11. 2	9. 0
360	0	16. 6	6. 02	9. 8	9. 1
400	0	16. 8	5. 22	9. 8	9. 1
0	18. 2	16. 1	15. 22	9. 8	9. 1

In this series the concentration of the arsenic was not carried above 400 p. p. m., for previous work had shown that the main stimulation occurs below this concentration. Furthermore the arsenic occurring in agricultural soils seldom exceeds 150 p. p. m., so it is likely that in agricultural soils it will never be found to exceed the quantity used in this work.

The results reported in the above table bring out some very interesting facts and show that the nitrogen-fixing organisms are very similar to the nitrifying organisms in so far as their relations to arsenic are concerned. The addition of 20 p. p. m. of sodium arsenate stimulates their action and 40 p. p. m. or more have a toxic influence. When the concentration of arsenic reaches 280 p. p. m., it stops all nitrogen-fixing activity. The toxic influence which becomes so very prominent above this concentration must be due entirely to the arsenic and not to the sodium ion, as Lipman and Sharp (1912) have added many times this

quantity of sodium in the form of sulphates, chlorids, and carbonates to the soil without retarding its nitrogen-fixing power.

The lead arsenate at the lower concentrations has no influence upon the nitrogen-fixing powers of the soil, but when the concentration reaches 80 p. p. m. a stimulating influence becomes quite perceptible. This continues until the concentration exceeds 200 p. p. m. Above this concentration the nitrogen fixed, within experimental error, is the same as that fixed in the untreated soil. It is interesting to note that the compound does not become toxic, even when the quantity added reaches 400 parts of arsenic per million parts of soil. This series shows a very close similarity to the nitrification series previously reported, and it is quite likely that part of the stimulating influence is due to the lead ion.

Paris green is toxic even in the lowest concentration used, and the toxicity increases as the quantity of Paris green added increases. This toxicity is due mainly to the copper ion. However, as was shown in the ammonification and nitrification work, the quantity of soluble arsenic present would be much higher where the Paris green was added than where the other compounds were used. The fact that no stimulation occurs in the Paris-green series points to the conclusion that the toxicity of the copper must increase much more rapidly than the stimulating influence of the arsenic. Yet it is quite possible that if a lower concentration of the substance had been taken a stimulation would have been noted.

Arsenic trisulphid stimulates in the lowest concentration tested and increases in stimulating influence until a concentration of 160 p. p. m. is reached. In concentrations above this its stimulating influence decreases. In concentration above 320 p. p. m. there is fixed no more nitrogen in the presence than in the absence of arsenic. But even at the highest concentration tested (400 p. p. m.) this compound exerts no tonic influence on the nitrogen fixers.

Zinc arsenite probably stimulates slightly in low concentrations, but aside from this it has little apparent influence on the nitrogen-gathering organisms. Had fresh soil been used in this series, greater stimulation would have been noted, as was found by later work.

The amount of nitrogen fixed in the untreated soil of the above series shows a marked variation. This is probably due to various factors, chief among which is the fact that the nitrogen-fixing powers of the soil with sodium arsenate, lead arsenate, and Paris green were made in the order named on the air-dried soil soon after it had been brought to the laboratory. In the case of the arsenic trisulphid and zinc arsenite the soil had been in the laboratory in an air-dried condition for about two months before the determinations were made, but each set of samples within each series was handled in exactly the same manner, and the samples are directly comparable within each set, as has been the case in the previous

discussion. In order to make those containing different forms of arsenic more nearly comparable with each other—that is, the lead arsenate with the arsenic trisulphid, etc.—the nitrogen fixed in the untreated soil has been taken as 100, and from this the ratio has been calculated with each of the concentrations of arsenic. This gives us more nearly comparable results, which are shown in figure 1.

Comparing these results with those obtained for the ammonification and nitrification series (Greaves, 1913c), we find a marked similarity existing between them. In all of the series there is a marked stimulation with all of the compounds except Paris green. The arsenic trisulphid

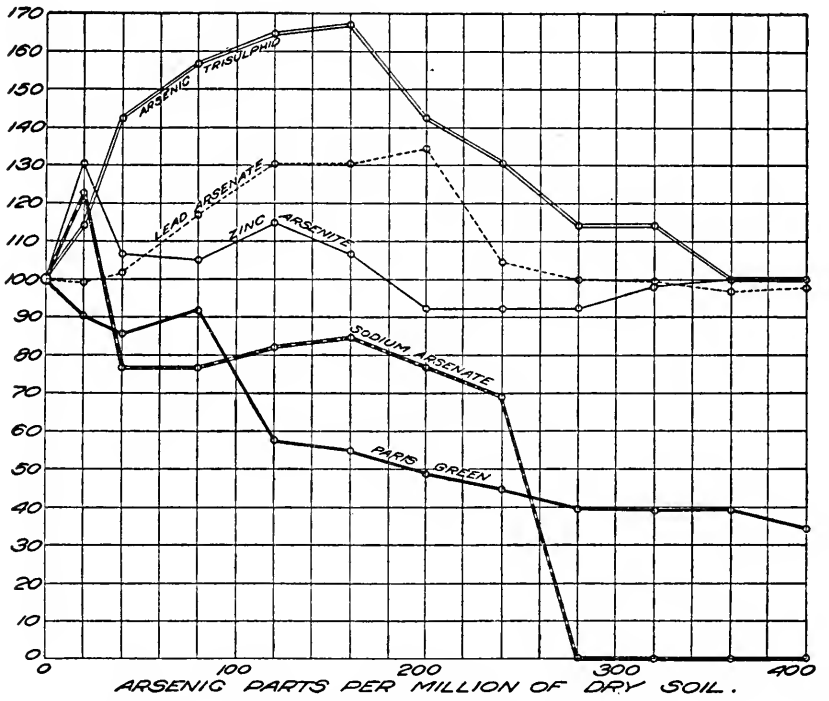


FIG. 1.—Graph showing the action of five compounds of arsenic on nitrogen fixation in dry soil. (Nitrogen fixed in untreated soil equals 100.)

stimulates growth much more in the nitrogen-fixing series than it does in the other series. The arsenic trisulphid has the greatest stimulating influence, followed in the order named by lead arsenate, zinc arsenite, and sodium arsenate. Paris green was the only compound tested which exerted no stimulating influence. It may be seen that the maximum stimulation was not obtained when equivalent quantities of arsenic in the various forms are applied to the soil. Hence, it seems possible that a relationship may exist among the various cases in the water-soluble arsenic found. In order to answer this, determinations were made of the water-soluble arsenic existing in the soil. The soil and arsenic,

together with 2 gm. of mannite, were placed in sterile tumblers, the water content made up to 18 per cent, and then incubated at 28°C. for 18 days. At the end of this period the soil was transferred by means of 1,000 c. c. of carbon-dioxid-free distilled water to large acid bottles. The mixture was left in these bottles, with occasional shaking, for 8 days, then filtered and the arsenic determined in an aliquot part (Greaves, 1913d). In another set the various forms of arsenic were mixed with 100-gm. portions of soil and 2 gm. of mannite and the water-soluble arsenic determined as above without incubation.

The results are given in Table II as milligrams of water-soluble arsenic occurring in 100 gm. of the soil both before and after the three weeks' incubation. Each reported result is the average of three or more closely agreeing determinations.

TABLE II.—*Quantity of water-soluble arsenic (in milligrams) in 100 gm. of soil before and after three weeks' incubation*

Treatment.	Lead arsenate.	Arsenic trisulphid.	Sodium arsenate.
Arsenic added	16. 00	16. 00	2. 00
Arsenic found before incubation	1. 04	. 14	1. 08
Arsenic found after incubation	1. 26	1. 42	1. 44
Average	1. 15	. 78	1. 26

The arsenic in each case became more soluble as bacterial activity progressed. This is especially marked in the soil containing arsenic trisulphid, which yielded 10 times the water-soluble arsenic after incubation that it did before. A remarkably close agreement is found to exist among the results obtained for water-soluble arsenic at the close of the incubation period, which shows that the maximum stimulating influence is obtained when soil contains between 10 and 15 p. p. m. of water-soluble arsenic. This is a quantity that exceeds that found in agricultural soil (Greaves, 1913b); hence, the influence of the arsenic occurring in soil must be to increase and not to retard nitrogen fixation. The maximum fixation varies with the form of arsenic applied. This is undoubtedly due, as will be pointed out later, to the elements accompanying the arsenic, which may have either a retarding or an accelerating influence upon the bacterial activity.

The finding of this marked stimulating influence of arsenic upon the nitrogen-fixing powers of soil raises a number of very interesting and important questions. Some of these are: (1) Does this stimulating influence exist in other soil or is there something inherent within this particular soil which makes its bacterial flora susceptible to the influence of arsenic? (2) Is the stimulating influence brought about by the retarding of injurious species or is it a direct stimulant to the soil organisms?

(3) Do the arsenic and arsenic compounds act as a source of energy to the nitrogen-fixing organisms or do they so influence the soil flora that it can utilize more economically the carbon compounds available? (4) What nitrogen-fixing organisms are there in the soil which are influenced by arsenic?

In order to find whether arsenic influences the nitrogen-fixing powers of other soils in a similar manner, three other soils were tested with and without arsenic. The soils vary greatly in chemical and physical composition. Soil A is a black loam of very light texture and, for an arid soil, high in nitrogen and humus. It is well supplied with phosphorus, potassium, and calcium carbonate and grew potatoes for 23 years. After this it was planted to oats for 2 years, and during the past 4 years has been planted in alfalfa. It has received some manure. Soil B is a sandy loam of much lighter color than soil A and contained much less humus and nitrogen, but an abundance of other elements. It has been cultivated for 28 years and during this time has been fallowed two summers. The remainder of the time it has been planted in wheat. Soil C is a heavy clay almost devoid of humus. The nitrogen is low, but the soil is well supplied with phosphorus, potassium, and calcium carbonate. While wet it is exceedingly sticky, and on drying it bakes like adobe. It has been tilled for 23 years, and during this time it has been fallowed for 3 years. The remainder of the time it has been in wheat. While it has received no manure during this time, it is still very productive. All of the soils are very fertile and well supplied with Azotobacter, and previous work has shown them to have high nitrogen-fixing powers.

The soils were all air-dried in the dark for 24 hours, ground in a mortar, sieved, weighed, and placed in sterile tumblers. Some were mixed with mannite and arsenic, others with mannite, while still others received only arsenic. They were all incubated in the regular manner, and the nitrogen determined as in the previous series. The results are given in Table III. Each reported result is the average of six closely agreeing determinations.

A marked stimulation is found in every case where the arsenic and mannite were applied to the soil, as compared with the results obtained where the mannite only was applied. The action of the various arsenical compounds follows the same order in each of these soils that it did in the first soil tested, being greatest with the lead arsenate and least with the sodium arsenate. The nitrogen fixed in the presence of arsenic but in the absence of mannite is usually considerably higher than that fixed in the presence of mannite and absence of arsenic. It would not be right to conclude from these results that the arsenic compounds furnish a source of energy to the nitrogen-fixing organisms, for these soils (Greaves, 1914, p. 456) have been found to fix appreciable quantities of nitrogen when incubated with an optimum moisture content without the addition of any carbon compound. It is likely that the arsenic makes the nitrogen-

gathering organism use more economically its usual source of carbon, which in the absence of mannite is probably the plant débris which has been slowly added to the soil. The belief that this is the case is strengthened by the fact that soil rich in organic matter (soil A) acts practically the same in the absence of mannite and presence of arsenic as it does when both arsenic and mannite are added to the soil. The clay soil (C), which is low in organic matter, acts about the same in the absence of arsenic as in the absence of mannite. It is interesting to note that in soils B and C the total fixation in the soil containing mannite plus that fixed by the soil containing arsenic approximates the total fixation in the series in which both arsenic and mannite are present.

TABLE III.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic*

Soil.	16 mgm. of arsenic, 2 gm. of mannite, added to soil.	16 mgm. of arsenic, no mannite, added to soil.	No arsenic, 2 gm. of mannite, added to soil.	Total of columns 2 and 3.
LEAD ARSENATE				
A	17.0	16.8	6.7	24.5
B	16.8	9.8	4.0	13.8
C	10.5	5.3	6.3	11.6
Average	14.7	10.6	6.0	16.6
ARSENIC TRISULPHID				
A	16.3	15.6	13.8	29.4
B	12.6	7.0	7.6	14.6
C	10.6	5.6	4.2	9.8
Average	13.1	9.4	8.5	17.9
SODIUM ARSENATE				
A	7.8	6.3	6.3	12.60
B	7.0	4.9	3.3	8.20
C	9.2	8.4	7.0	15.40
Average	8.0	6.5	5.5	12.0

In all of the tests so far reported the incubation period has been 18 days. Longer periods of incubation may give results very different from those so far obtained, for the stimulating influence of arsenic may be of short duration, and we may find later a slowing up of the reaction, or, inasmuch as we are dealing with the algebraic sum of many reactions which are taking place in the soil, we may find it to be negative. An attempt was made to determine this by the following experiment: 100-gm. portions of the high-humus soil (A) were mixed with 0.0728

gm. of lead arsenate and the moisture content made up to 18 per cent and then weighed. One-half of the samples thus prepared were sterilized in the autoclave and all of them placed in an incubator at a temperature of from 28° to 30° C. The moisture was made up weekly to its initial content. Beginning at the end of 20 days, six samples, three autoclaved and three not autoclaved, were used for the making of duplicate total-nitrogen determinations. The average excess of nitrogen in the unsterilized soil over that in the sterilized is given in Table IV.

TABLE IV.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil containing 0.0728 gm. of lead arsenate*

Time incubated.	Nitrogen.	Time incubated.	Nitrogen.
Days.	*Mgm.*	*Days.*	*Mgm.*
20	12.32	96	1.00
30	−12.40	162	3.80
44	−16.40	172	6.20
66	−8.20		

The greatest quantity of nitrogen was obtained at the end of 20 days. During the next 10 days, however, 24.72 mgm. of combined nitrogen disappeared. During the next 14 days there was a loss of only 4 mgm. From this time on there was a gradual increase in the amount of combined nitrogen found within the soil up to the end of the experiment, but even after 172 days' incubation there was less nitrogen in the soil than there was at the end of 20 days.

The great loss of nitrogen can not be entirely charged up to the arsenic added, for other workers (Ashby, 1907; Hoffmann and Hammer, 1910, p. 164) have noted, when working with impure cultures, a loss of nitrogen on prolonged incubation in the absence of arsenic. The loss is probably due to the soil's becoming compact, with the production of anaerobic conditions. This, assisted by the protozoa (Miller, 1914, p. 217), which appropriate too large a share of the limited supply of oxygen in the soil, prevents entirely the activity of the aerobic nitrogen-fixing organisms and greatly stimulates the activity of the denitrifying organisms of the soil. This can, however, only partly account for the phenomena; otherwise there would be a continual decrease in the nitrogen as the soil became more compact.

The fact that aeration plays a considerable part in the reaction is brought out by the following experiment, which differs from the preceding only in that the soil was thoroughly stirred, thus aerating it each time before making up the moisture content. The results of this experiment are given in Table V.

TABLE V.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of aerated soil with and without the addition of arsenic after different periods of incubation*

Days incubated.	Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate.	Nitrogen fixed in untreated soil.	Days incubated.	Nitrogen fixed in soil containing 0.0728 gm. of lead arsenate.	Nitrogen fixed in untreated soil.
20	8. 12	2. 58	66	9. 38	14. 00
30	5. 88	3. 92	96	4. 90	2. 52
44	8. 26	3. 78	162	2. 80	4. 20

These results show conclusively that it was the lack of air in the former series which caused such great losses of nitrogen and that they could in no way be attributed to the arsenic added. This series was stirred but once a week and after the stirring the moisture content was made up to the optimum so that the soil became quite compact. It is quite likely that greater care in the aeration of the soil would have reduced very materially the loss of nitrogen which was observed in this series. In the first stages of the experiment the soil containing arsenic gained the greater quantity of nitrogen, while in the later stages the soils containing no arsenic were the highest. If, however, an average of the quantity found in each soil is taken, it will be found to be considerably higher in the soil containing arsenic than in the other.

It was thought that some of the questions referred to in the first part of this article could be answered more readily with the solution method than with soil. For this reason a series was incubated using a solution of the following composition:

Dibasic potassium phosphate (K_2HPO_4).... 0.2 gm.
Magnesium sulphate ($MgSO_4$)..... .2 gm.
Calcium chlorid ($CaCl_2$)..... .02 gm.
Ferric chlorid (Fe_2Cl_6)..... 1 drop (10 per cent solution).

This was made up to 1,000 c. c. with tap water and distributed in 100 c. c. portions into 750 c. c. Erlenmeyer flasks. One gm. of calcium carbonate was added to each, and the flasks were then sterilized and inoculated. One series was inoculated with *Azotobacter vinelandii*. This was done by making a suspension in sterile tap water of the organism and adding 5 c. c. of this suspension to each flask. In the other series the inoculating medium was 10 gm. of soil. The solutions were incubated at 28° to 30° C. for 18 days, and then the nitrogen determined in the manner previously outlined. The results are given in Table VI and are reported as milligrams of nitrogen fixed in 100 c. c. of the solution. Each reported result is the average of three closely agreeing determinations.

TABLE VI.—*Quantity of nitrogen (in milligrams) fixed in 100 c. c. of nutritive solution with and without the addition of arsenic*

Treatment.	Inoculated with *Azotobacter vinelandii*.	Soil +0.0728 gm. of sterilized lead arsenate.	Soil +0.0728 gm. of unsterilized lead arsenate.
Nutritive solution + 1.5 gm. of mannite.....	14.12	15.16	15.77
Nutritive solution + 1.5 gm. of mannite and 0.0728 gm. of lead arsenate.....	0	14.79	13.72
Nutritive solution + 0.0728 gm. of lead arsenate.....	0	1.45	.52
Nutritive solution + 1.5 gm. of mannite and 0.0272 gm. of arsenic trisulphid.....	.5	5.98	2.05
Nutritive solution + 0.0272 gm. of arsenic trisulphid	0	.28	.08

After the first series had been completed, it was thought possible that the heat in the autoclave had changed the solubility of the arsenical compounds and that this was the reason there was no fixation in the solution with arsenic. For this reason analyses were made of the soluble arsenic in 100 c. c. of the nutritive solution containing arsenic both before and after autoclaving. The determinations were made as previously outlined. The lead arsenate yielded 0.91 mgm. of soluble arsenic before autoclaving and 0.85 mgm. after autoclaving. The arsenic trisulphid yielded 0.40 mgm. before autoclaving and 0.42 mgm. after autoclaving.

The results indicate conclusively that the toxicity of the compound is not due to a difference in the solubility of the compound produced by the heat. In order to make sure of this, a series was arranged in which the arsenic was added just before inoculation and after the solution had been autoclaved. These results are given in the last column of Table VI and are slightly lower than those previously obtained with the arsenic. The *A. vinelandii* fixed no nitrogen in the presence of the arsenic. Even where the soil was used as the inoculating medium, the lead arsenate retarded nitrogen fixations to a certain extent. The toxic influence of the arsenic sulphid is very pronounced. These results show the care which must be used in drawing conclusions from the Remy-solution method as to what is to be expected in soils. They greatly strengthen the contention of Jönsson (1896) that the fact that Nobbe (1884) found arsenic solutions to be toxic to seedlings in water culture and concluded that arsenic, even in small quantities, is extremely toxic to plants does not indicate that these solutions will be toxic when in the soil. The results herein reported show arsenic to be extremely toxic to nitrogen-fixing organisms while in solution, but the same concentration in the soil is not only devoid of toxicity but acts as a powerful stimulant. This therefore establishes for the bacteria what Kanda (1904, p. 16) found to be true for the higher plants—namely, that dilute solutions of sub-

stances may be toxic when used in water culture, but that the same quantities when placed in the soil may act as stimulants.

The results reported for *A. vinelandii*, when considered in connection with those obtained for the soil, make very problematic the part played by Azotobacter, especially *A. vinelandii*, in these soils. The exact mode of action of the arsenic also remains a question. For these reasons the soil used in the first series was plated and the main nitrogen-fixing organisms isolated. Three types of Azotobacter were obtained. These have been designated Azotobacter A, Azotobacter B, and Azotobacter C. Azotobacter A has a nitrogen-fixing power of 6.86 mgm. of nitrogen per gram of mannite in Ashby solution, Azotobacter B a nitrogen-fixing power of 5.00 mgm., and Azotobacter C a nitrogen-fixing power of 6.44 mgm. of nitrogen.

The preceding results have shown that little information of value can be obtained by the solution method. Therefore another series was planned in which 100-gm. portions of the soil used in the first series were weighed into covered sterile tumblers and autoclaved at a temperature of 120° C. for 30 minutes, cooled, and the moisture content made up to 18 per cent. The soil was then inoculated with the various organisms which had been isolated. The soil portions were incubated for 18 days, the moisture content kept constant, and then the total nitrogen determined. Sterile blanks were incubated and analyzed as checks. Each reported result is the average of four or more closely agreeing determinations, so that the analytical error has been reduced to a minimum. The results are given in Table VII.

TABLE VII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic and inoculated with various nitrogen-fixing organisms*

Inoculating organism.	Milligrams of nitrogen fixed in 100 gm. of soil treated with—		
	2 gm. of mannite, 0.0728 gm. of lead arsenate.	2 gm. of mannite, no arsenic.	0.0728 gm. of lead arsenate, no mannite.
Azotobacter A	15.60	21.70	3.01
Azotobacter B	24.15	14.70	8.80
Azotobacter C	18.20	18.20	4.90
Azotobacter A and B	26.31	22.05	5.81
Azotobacter A, B, and C	18.40	17.70	6.65

The results reported above show for each organism a fixation much higher in the soil than was found in the solution. The results without arsenic, but with mannite, are as high as are reported in Table I with both mannite and arsenic combined, a fact which would seem to indicate that arsenic acts upon injurious species. This, however, does not account for the entire phenomenon, for we find in this series a very small fixation of nitrogen in the absence of mannite and presence of arsenic, while in

the ordinary soil with its mixed flora as great a fixation was obtained in the presence of arsenic as in the presence of only mannite. This probably indicates that some of the stimulation is due either to the fact that the arsenic acts upon allied species which are gathering carbon that can be used by the Azotobacter, or else to the fact that some species, possibly the cellulose ferments, are stimulated so that they render available to the Azotobacter the carbon-carrying compounds of the soil faster in the presence of arsenic than in its absence. Only one of the organisms isolated, Azotobacter B, is directly stimulated by arsenic. The stimulation, however, is very large in this case. It also fixes large quantities of nitrogen in the presence of arsenic and absence of mannite. These results are complicated by the carbonaceous material which occurs in the soil. For this reason a series similar to the above was incubated, using silica sand in place of the soil. The silica used was devoid of organic matter and had the following composition:

	Per cent.
Silicon dioxid (SiO_2)	97.5
Ferrous oxid (FeO)	.1
Aluminum oxid (Al_2O_3)	1.7
Calcium oxid (CaO)	.2

One-hundred gm. portions of this were sterilized in covered tumblers, and to each was added 1 gm. of calcium carbonate and 18 c. c. of sterile distilled water containing 0.02 gm. of potassium phosphate, 0.02 gm. of magnesium sulphate, and 0.002 gm. of calcium chlorid. The tumblers were inoculated with the various nitrogen-fixing organisms incubated with a constant moisture content at 28° C. for 18 days, and then the nitrogen determined as in the previous series. They were all compared with sterile blanks. The results are given in Table VIII as milligrams of nitrogen fixed in 100 gm. of sand. Each reported result is the average of six or more closely agreeing determinations.

TABLE VIII.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of quartz sand with and without arsenic*

Inoculating material.	Sand and Ash-by solution, +0.0728 gm. of lead arsenate.	Sand and Ash-by solution, no arsenic.	Sand and Ash-by solution, no mannite, +0.0728 gm. of lead arsenate.
10 c. c. of soil extract	19.60	10.50	4.70
Azotobacter A	17.01	22.61	0
Azotobacter B	13.84	12.60	0
Azotobacter C	15.10	16.80	0

Qualitatively, the above results are the same as those obtained with the soil. Azotobacter B was the only one of the three organisms stimulated by the arsenic. Where the mixed flora were used, the stimulation was very marked, but the fixation in the absence of arsenic where either Azotobacter A or Azotobacter C was used is about the same as that

obtained in the presence of arsenic where the soil extract was used. This fact would seem to indicate that the main stimulation brought about by arsenic is due to its action upon injurious species. The results obtained in the presence of arsenic and absence of mannite indicate that the Azotobacter can not use the arsenic as a source of energy. The small fixation where the soil extract was used may be due to the nitrogen-fixing organisms obtaining a small quantity of carbon compounds from algæ which may have grown in the complex flora.

The results given in Table VII pointed strongly to the conclusion that the stimulating influence of the arsenic was due in part to an indirect action upon the nitrogen-fixing organisms, possibly an action which it exerts upon the cellulose ferment. A series was therefore arranged in which the cellulose ferments were used in connection with the Azotobacter.

In this series 100-gm. portions of the high humus soil (A) were placed in covered tumblers and sterilized in the autoclave and then treated as in Table IX. The Azotobacter was inoculated into 100 c. c. of Ashby solution. After three days the solution was thoroughly shaken and 5 c. c. of the solution were added to the sterile soil. The cellulose ferment was added by making a suspension of the organism in sterile distilled water and adding 5 c. c. of this to the soil. The moisture content was made up to 18 per cent and incubated for 18 days. Six samples of each were used, so that the results reported are the averages of six closely agreeing determinations. The results are given in Table IX. The cellulose ferments used were *Bacillus rossicus*, isolated by Kellerman, McBeth, and others (1913) from Geneva (N. Y.) soils, and *Pseudomonas effusa*, isolated by the same investigators from the soils used in this work.

TABLE IX.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil with and without arsenic in the presence and absence of cellulose ferments*

Treatment.	Nitrogen gained.
Azotobacter chroococcum	14. 70
Azotobacter chroococcum, 0.0728 gm. lead arsenate	14. 28
Azotobacter chroococcum, *Bacillus rossicus*	26. 18
Azotobacter chroococcum, *Bacillus rossicus*, 0.0728 gm. of lead arsenate	28. 00
Azotobacter chroococcum, *Pseudomonas effusa*	13. 30
Azotobacter chroococcum, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate	22. 68
Azotobacter B	14. 46
Azotobacter B, 0.0728 gm. of lead arsenate	21. 00
Azotobacter B, *Bacillus rossicus*	15. 20
Azotobacter B, *Bacillus rossicus*, 0.0728 gm. of lead arsenate	19. 60
Azotobacter B, *Pseudomonas effusa*	14. 00
Azotobacter B, *Pseudomonas effusa*, 0.0728 gm. of lead arsenate	21. 00

In this series, as in the previous series in which *A. chroococcum* was used, it did not fix as much nitrogen in the presence of arsenic as it did in the absence of it. *A. chroococcum* fixes nearly twice the quantity in the

presence of *B. rossicus* as in its absence, and when arsenic is added to the two there is an even greater fixation. This is also the case with *P. effusa;* measured in terms of the increased nitrogen fixed by *A. chroococcum*, it may therefore be safely concluded that both of the cellulose ferments are stimulated by lead arsenate.

The Azotobacter B differs from the *A. chroococcum* in that it is directly stimulated by the arsenic, but is not as greatly helped by the cellulose ferment. In this case the lead arsenate greatly stimulates the activity of the cellulose ferments, and the stimulating influence is much greater with *P. effusa*, the normal habitat of which is this soil, than it is with *B. rossicus*. Hence, from this work it is safe to conclude that the cellulose organisms, so far as arsenic is concerned, obey the same laws as do the ammonifying, nitrifying, and nitrogen-fixing organisms of the soil.

It has been noted throughout all of this work that the soil taken direct from the field was stimulated to a much greater extent by the arsenical compounds than was the air-dried soil. Furthermore, it was noted that the soil which had stood in the laboratory for a great length of time was stimulated only very slightly by arsenic. For these reasons a series of experiments was planned to throw more light upon this substance or organism which disappears on drying.

Fred (1911) has suggested the use of filter paper for the separation of the protozoa. Later this has been shown by Kopeloff and others (1915) to be quite effective. Using this suggestion, 100-gm. portions of soil were placed in tumblers. To half of them was added 0.0728 gm. of lead arsenate, and the mixture was autoclaved until free from bacterial life. They were all inoculated with 10 c. c. of a solution obtained by shaking 100 gm. of soil in 1,000 c. c. of sterile water and then filtering through three thicknesses of a fine grade of quantitative filter paper, after which they were incubated and nitrogen determined as in the previous set. The results are given in Table X as milligrams of nitrogen per 100 gm. of soil. All results are averages of six determinations made on that number of incubated samples.

TABLE X.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of sterile soil inoculated with filtered soil extract, with and without arsenic*

Time incubated.	0.0728 gm. of lead arsenate.	No lead arsenate.
Days.		
20	16.10	14.70
30	3.08	2.52
44	2.80	.30
66	1.94	.14
96	.28	.28
162	.78	.85

It probably would have been better if in every case untreated soil could have been incubated with the variously treated soil, but this so greatly increased the number of determinations that it was not thought advisable. Furthermore, all the work has been done on the high-humus soil, A, without the addition of any carbohydrate, and repeated determinations have shown that the arsenic more than doubles the nitrogen fixed in the soil in 20 days, so that the absence of the stimulation can be safely attributed to the treatment. In the above results, it is readily seen that the soil extract on passing through filter paper loses to a very great extent its power of being stimulated by arsenic. Hence, it is safe to conclude that the main stimulating influence of arsenic upon nitrogen fixation is due to its suppressing something which is found in the soil and which is removed by the filter paper.

That this factor is to a great extent the same as is removed by heat is shown by the results reported in Table XI. The arrangement of this series of experiments was as follows: 100-gm. portions of the soil were weighed into covered tumblers. To one-half of the set was added arsenic—0.0728 gm. to each 100 gm. of soil. The tumblers were all carefully sterilized and half of them were placed in the incubator in the sterile condition. To the others was added a soil extract prepared by shaking one part of soil with two parts of sterile distilled water for three minutes. After standing for about five minutes the liquid was decanted and 10 c. c. portions of this were used to inoculate the soil. Before inoculating, this extract was placed in thin-walled test tubes in 10 c. c. portions and then held at the required temperature for exactly 15 minutes before adding to the soil. The moisture content was made up to 18 per cent and the whole incubated for 20 days. Each reported result is the average of six closely agreeing determinations.

TABLE XI.—*Quantity of nitrogen (in milligrams) fixed in 100 gm. of soil, with and without arsenic, inoculated with soil extract*

Temperature of soil extract (°C.).	0.0728 gm. of lead arsenate added.	No arsenic added.
Room	8.77	5.11
50	9.24	9.00
55	14.28	14.14
60	12.60	16.38
65	13.85	14.42
70	12.18	13.02
75	12.88	11.34
80	13.44	12.66
85	11.54	10.36

The heating of the soil extract to a temperature of 55° C. for 15 minutes changes the soil so that it is no longer stimulated by arsenic. The heating of the soil extract to a higher temperature stimulates its nitrogen-

fixing properties. It is not, however, increased by the addition of arsenic. Hence, it would appear as if the substance which is suppressed by the arsenic is very thermolabile and is easily injured by drying, for it has been repeatedly brought to our attention that the drying of the soil prevents the arsenic from greatly stimulating its nitrogen-fixing properties. Harden and Young (1911, p. 72; 1906) have shown that the addition of arsenates to a yeast-juice sugar solution greatly accelerates the rate of fermentation of such a mixture. The close analogy existing between the chemical properties of phosphorus and arsenic led to the idea that possibly the arsenic replaced the phosphorus in the reaction characteristic of phosphorus, but they found that this is not the case, for while the arsenic has an optimum concentration, as has the phosphorus, there was no direct relationship between the amount of arsenate added and the extra amount of fermentation, the arsenic in this way acting more like a catalyzer than does the phosphorus. Furthermore it was shown that fermentation can not proceed in the absence of phosphorus, even though there be present either arsenates or arsenites. The arsenic acts mainly as a liberator of the phosphorus from the hexosephosphates and does not of itself enter into the vital reactions of the cell as does the phosphorus.

These facts make it likely that a similar action may be exerted by the arsenic upon the bacteria. For these reasons a series of experiments was arranged in which the phosphorus had been replaced by arsenic. These were carried on in the nitrogen-free quartz sand. To each 100 gm. of the sand there was added the quantity of carefully tested nutrient without phosphorus found in 100 c. c. of Ashby's solution. To one-half of them was added the phosphorus, while to the other half there was added 0.0728 gm. of lead arsenate. They were each inoculated with 1 c. c. of a soil extract and then incubated the regular length of time. The nitrogen determinations were made on them and sterile blanks with the following results: When incubated with complete Ashby's solution and 0.0728 gm. of lead arsenate, 100 gm. of sand fixed 11.62 mgm. of nitrogen. Similar samples without phosphorus but with arsenic fixed 0.03 mgm., while without phosphorus or arsenic there was fixed 0.01 mgm. of nitrogen. The results for the set with the complete nutritive media show that sufficient of the soil extract was taken to get the nitrogen-fixing organism, and the results without phosphorus show that there was not sufficient phosphorus in the 1 c. c. of soil extract to furnish phosphorus for the organisms. These results show conclusively that arsenic can not replace phosphorus in the vital activities of the nitrogen-fixing organisms of the soil, and establish for this set of organisms what Stoklasa (1897) has established for the higher phanerogams, Molisch (Lafar, 1911, p. 37) for algæ, Günther (1897) for the molds, and Harden and Young (1906) for the yeasts.

There is still the possibility that the arsenic liberates the phosphorus from its insoluble compounds in the soil and thus makes it more available to the micro-organisms. If this be the case, one would think that the addition of soluble phosphates to the soil investigated would increase its nitrogen-fixing powers. Experiments, however, did not bear out this assumption, for just as large a quantity of nitrogen was fixed in the absence of the soluble phosphate as in its presence. This was probably due to the fact that the soil under investigation was well supplied in the natural condition with soluble phosphorus. But that the arsenic did have an influence upon the solubility of the phosphorus of the soil was shown by the following experiment: 100-gm. portions of the soil were placed in covered tumblers. Of these, 24 received 0.0728 gm. of lead arsenate each, while the other 24 received none. The moisture was made up to 18 per cent and incubated for 20 days. At the end of this time the water-soluble phosphorus was determined in 12 of the treated and 12 of the untreated soils by extracting with 500 c. c. of distilled water and determining the phosphorus in the extract (Greaves, 1910). As an average of the 12 closely agreeing determinations of the soil treated with arsenic there was obtained 0.59 mgm. of water-soluble phosphorus, while the untreated soils yielded 0.52 mgm. This is a slightly greater quantity in the arsenic-treated soil than in the untreated, which is probably due to the fact that more of the phosphorus had been changed in the body of the soil organisms to nucleoproteins or phosphoproteins. That this is the correct interpretation is shown by the results obtained from the remaining samples. Twelve of these samples, six with and six without arsenic, were digested for six hours with 100 c. c. of 12 per cent hydrochloric acid and the phosphorus determined in the filtrate. The other samples were ignited and the phosphorus extracted by the 12 per cent hydrochloric acid determined. The average of the results thus obtained is given in the tabular form below:

SAMPLES NOT IGNITED:	
Soil with arsenic	105.6 mgm. of phosphorus.
Soil without arsenic	100.0 mgm. of phosphorus.
Excess of acid-soluble phosphorus in soil with arsenic	5.6 mgm. of phosphorus.
SAMPLES IGNITED:	
Soil with arsenic	107.7 mgm. of phosphorus.
Soil without arsenic	100.8 mgm. of phosphorus.
Excess of acid-soluble phosphorus in soil with arsenic	6.9 mgm. of phosphorus.

This would give by the Schmoeger method 2.10 mgm. of organic phosphorus in the arsenic-treated soil, while in the untreated soil there was

only 0.80 mgm. of organic phosphorus. This excess of organic phosphorus could not have come from the water-soluble phosphorus, as there was a difference of only 0.07 mgm. in the two soils; hence, it must be concluded that the arsenic increases the solubility of the phosphorus. This, however, may be due either to a direct interchange between the insoluble phosphorus of the soil and the arsenic or to its action upon bacteria, which causes them to become more active in growth and formation of various acids which act upon the insoluble phosphates of the soil, rendering them soluble.

GENERAL CONSIDERATIONS

The data reported prove conclusively that the arsenical compounds, with the single exception of Paris green, stimulate the nitrogen-fixing organisms of the soil and that this influence varies qualitatively but not quantitatively with the various soils. The results also bring out the fact that both the anion and the cation of the compounds have a marked influence upon the growth of the organisms. With some compounds both the anion and cation act as stimulants, while with others one stimulates and the other is markedly toxic. It is likely that little or no influence is exerted upon the nitrogen-gathering organisms by the sodium (Lipman and Sharp, 1912), and that the stimulating influence noted with dilute solutions and the toxic influence exerted with more concentrated solutions are due entirely to the arsenic. It is quite likely that the stimulating influence which Rivière and Bailhache (1913) have found sodium arsenate to have upon wheat and oats is an indirect effect which is exerted upon the bacterial flora of the soil and which in turn influences the yield of the various grains.

Both the anion and cation undoubtedly act as stimulants in the lead arsenate. Stoklasa (1913) has shown that lead when present in soil stimulates the growth of higher plants. This he (1911) ascribes to the catalytic action of these elements on the chlorophyll. The results herein reported, together with those previously published (Greaves, 1913a), indicate that it is due to the influence of the compounds upon the biological transformation of the nitrogen in the soil. The fact that the lead plays no small part in the stimulating influence is borne out by the work of Lipman and Burgess (1914), who found lead to stimulate nitrifying organisms.

Paris green is toxic to the nitrogen-fixing organisms in the lowest concentration tested. This is due to the copper and not to the arsenic, as it is well known that the copper ion is a strong poison to many of the lower plants. Brenchley (1914) found it to be toxic to higher plants when present in water to the extent of 1 part in 4,000,000,000. Although Russell (1912, p. 47) states that it is not as toxic in soil as in water, Darbishire and Russell (1905) found it to be toxic in soils, and they failed to get a stimulating influence with it. Montemartini (1911)

has noted a stimulation with copper sulphate when used in dilute solutions. This, however, may have been due to the anion and not to the cation, as sulphates do stimulate plants by their action on insoluble constituents of the soil (Greaves, 1910, p. 298). The same interpretation could be placed upon the results obtained by Lipman and Wilson (1913) and also those reported by Voelcker (1913), in which they noted a stimulation with copper salts. Clark and Gage (1906) have found that very dilute solutions of copper have an invigorating influence upon bacterial activity. In order that the stimulation may be noted the copper must be present in small quantities. Jackson (1905) found that 1 part of copper sulphate in 50,000 parts of water killed *Bacillus coli* and *B. typhosus*. Kellerman and Beckwith (1907) found that the common saprophytic bacteria are more resistant to copper than is *B. coli*. There is considerable evidence (Lipman and Burgess, 1914; Greaves, 1913a, p. 8) that copper stimulates the ammonifying and nitrifying organisms of the soil, but these results show the nitrogen-fixing organisms of the soil to be very sensitive to copper, and if it does act as a stimulant it must be in extremely dilute solutions. The toxicity of the copper in the Paris green is great enough in the dilution of 10 parts in 1,000,000 to offset the great stimulating influence of the arsenic in combination with it.

The very marked stimulating influence noted where the arsenic trisulphid is used is very probably due to both the arsenic and the sulphur. Demolon (1913) attributed much of the fertilizing action of sulphur to its action upon bacteria, and Vogel (1914) found that sulphur decidedly increased the activity of the nitrogen-fixing organisms. The results which Russell and Hutchinson (1913, p. 173) obtained with calcium sulphid are interesting in this connection. They found that after 30 days there were five times as many organisms in the soil to which calcium sulphid had been added as in the untreated soil, and the yield of ammonia and nitrates in this time was one-third greater in the treated soil than in the untreated soil. This, in turn, reacts upon the crop harvested, as shown by Shedd (1914, p. 595).

The first part of the curve (fig. 1) for the zinc arsenite nearly coincides with that of the sodium arsenate, but the zinc arsenite stimulates in greater concentrations than does the sodium arsenate. This is partly due to the difference in solubility of the two compounds, but there is another factor which enters, and that is that the zinc also acts as a stimulant. Latham (1909) found that small quantities of zinc stimulated algæ. The same results have been obtained by Silberberg (1909) in working with higher plants. Ehrenberg (1910) concludes that zinc salts are always toxic when the action is simply on the plant, but that they may lead to increased growth through some indirect action on the soil. He found that zinc stimulated plant growth in soils, but when the soil was sterilized the zinc became toxic. Lipman and Burgess (1914, p. 133)

have shown that it does stimulate the nitrifying organisms and that the influence is shown by the yield obtained from such soils (Lipman and Wilson, 1913). The great variation in the results reported by the various investigators for zinc, arsenic, and lead is probably due to the fact that it modifies the bacterial flora of the soil, and when heated soil or water cultures are used a different result is noted. This, however, is not the only factor which enters, for these results show a marked difference in soil and in water. The lead arsenate stimulates the nitrogen-fixing organisms when placed in soils but becomes very toxic to the same organisms when placed in nutritive solutions.

The difference is due in part to the adsorption of the soil, but in this case we would have to attribute it to the silica compounds of the soil, for the nitrogen-fixing organisms are stimulated by arsenic in quartz sand

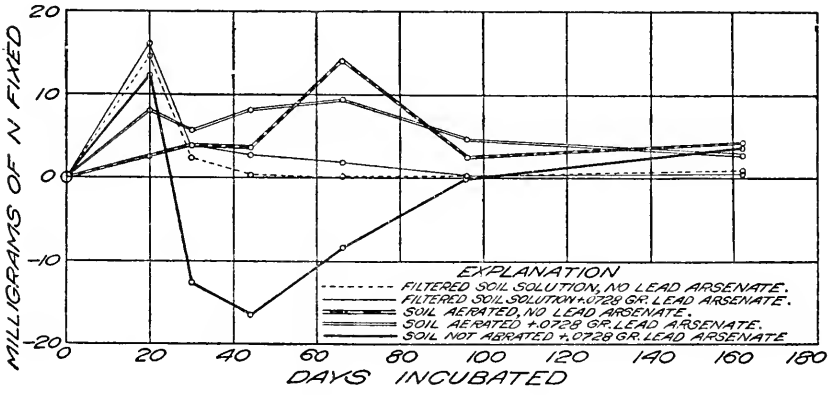


FIG. 2.—Graph showing the effect of aeration on the nitrogen-fixing activity of soil containing compounds of arsenic.

free from organic colloids. In this case the arsenic becomes concentrated at the surface, layers of the silica leaving the inner part of the water film comparatively free from arsenic, in which the micro-organisms multiply and carry on their metabolic processes. This being the case, one should, and probably could, find a water solution weak enough to stimulate bacteria. A great difference, however, between the solution and the sand-culture method is the greater aeration in the latter than in the former. That the aeration of a cultural medium does play a great part in determining the activity of the nitrogen-fixing powers of a soil is very strikingly brought out in figure 2. The graphs in this figure are made from the data given in Tables IV, V, and X.

It is remarkable how the aeration of the soil or the filtering of the soil extract can prevent the great loss of nitrogen which is noted at first in the unaerated soil. This can not be attributed directly to the denitrifying organisms; otherwise it would not be removed by filtration. The graphs

also bring out the fact that the addition of arsenic and the filtering of the soil only shift for the time the equilibrium within the soil, and later it tends to regain its old equilibrium. This is a condition which coincides well with what one would expect if the limiting element were some other microscopic forms of life. The filter would not separate them quantitatively, and it is possible that the arsenic has only a selective influence. Later, many of the organisms become accustomed to its presence; or, what is more likely, the arsenic becomes fixed (McGeorge, 1915) within the soil.

That this limiting factor is a thermolabile body is brought out more clearly in figure 3, which is made from the data reported in Table XI.

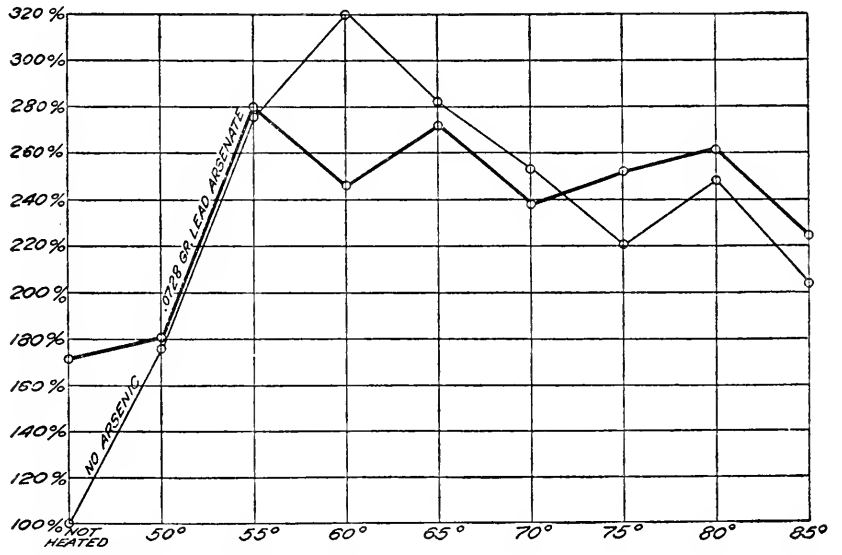


FIG. 3.—Graph showing the effect of heat on the nitrogen-fixing power of soil treated and not treated with arsenic.

The quantity of nitrogen fixed by the unheated soil receiving no arsenic has been taken as 100 per cent, and the heated soil with and without arsenic is compared with this.

The heating of the soil extract to 50° C. for 15 minutes has exactly the same influence measured in terms of nitrogen fixed as does 0.0728 gram of lead arsenate. The stimulating influence of heat is noted even in the presence of arsenic and reaches its maximum effect in the absence of arsenic at 60°, while in the presence of arsenic at 65° above these temperatures there is a decline in the nitrogen fixed. But even the soil inoculated with solutions which had been heated to a temperature of 85° fixed nitrogen; or at least there is more nitrogen accumulated in such soil than in that inoculated with the untreated soil solution. The results indicate that many of the organisms which take part in the gathering of nitro-

gen in this soil are very resistant to heat. It is also significant that the greatest stimulating influence is exerted in soil which had been inoculated with solutions heated just above what Cunningham and Löhnis (1914) found to be the thermal death point of soil protozoa.

The data presented in this paper, together with these presented in former publications, make it possible to compare the sensitiveness of the ammonifying, nitrifying, and nitrogen-fixing organisms toward the various arsenical compounds. Figure 4 represents the percentage of activity of the various classes of organisms in the presence of 400 p. p. m. of arsenic in the form of the various arsenical compounds. The untreated soil has been taken in every case as 100. The ammonifying organisms are retarded more by the lead arsenate than the nitrogen-fixing or nitrifying organisms. The latter two are influenced in nearly the same way by this concentration of lead arsenate. All three types of organisms are influenced in the same order by the arsenic trisulphid, while with the zinc arsenite the nitrogen-fixing and nitrifying organisms act about normally in concentrations of 400 p. p. m. of arsenic, but the ammonifiers are greatly depressed. Paris green stimulates the nitrifiers, but greatly depresses the other types of organisms. The results, with the exception of copper, show that the nitrifying and nitrogen-fixing organisms are very similar.

In figure 5 are shown graphically the quantities of arsenic in the form of various arsenicals which are required by the different organisms to give the greatest stimulation.

It has been shown that stimulation within a specific group of organisms varies with the quantity of water-soluble arsenic and the stimulating influence of the electropositive ion associated with the arsenic. But when we examine stimulation by these substances with different groups of organisms, we find a marked difference which can not be attributed to solubility but must be due to a physiological difference existing in the various organisms; for instance, the nitrogen-fixing organisms require 200 p. p. m. of arsenic in the form of lead arsenate for the greatest stimulation, while the nitrifiers and ammonifiers require much smaller quantities. For maximum stimulation with arsenic trisulphid the nitrogen-fixing organisms require the greatest concentration, followed by the nitrifying and ammonifying organisms in the order given. Zinc arsenite, on the other hand, has to be present in large quantities for a maximum stimulation of the nitrifying organisms, while very small quantities give a maximum stimulation with the other two groups of organisms. Practically the same order is followed by the organisms in the presence of sodium arsenate and Paris green, there being, however, this significant difference, that neither the ammonifiers nor the nitrogen-fixing organisms are stimulated in any concentration by the presence of copper, and it is quite possible that the same holds for the nitrifying

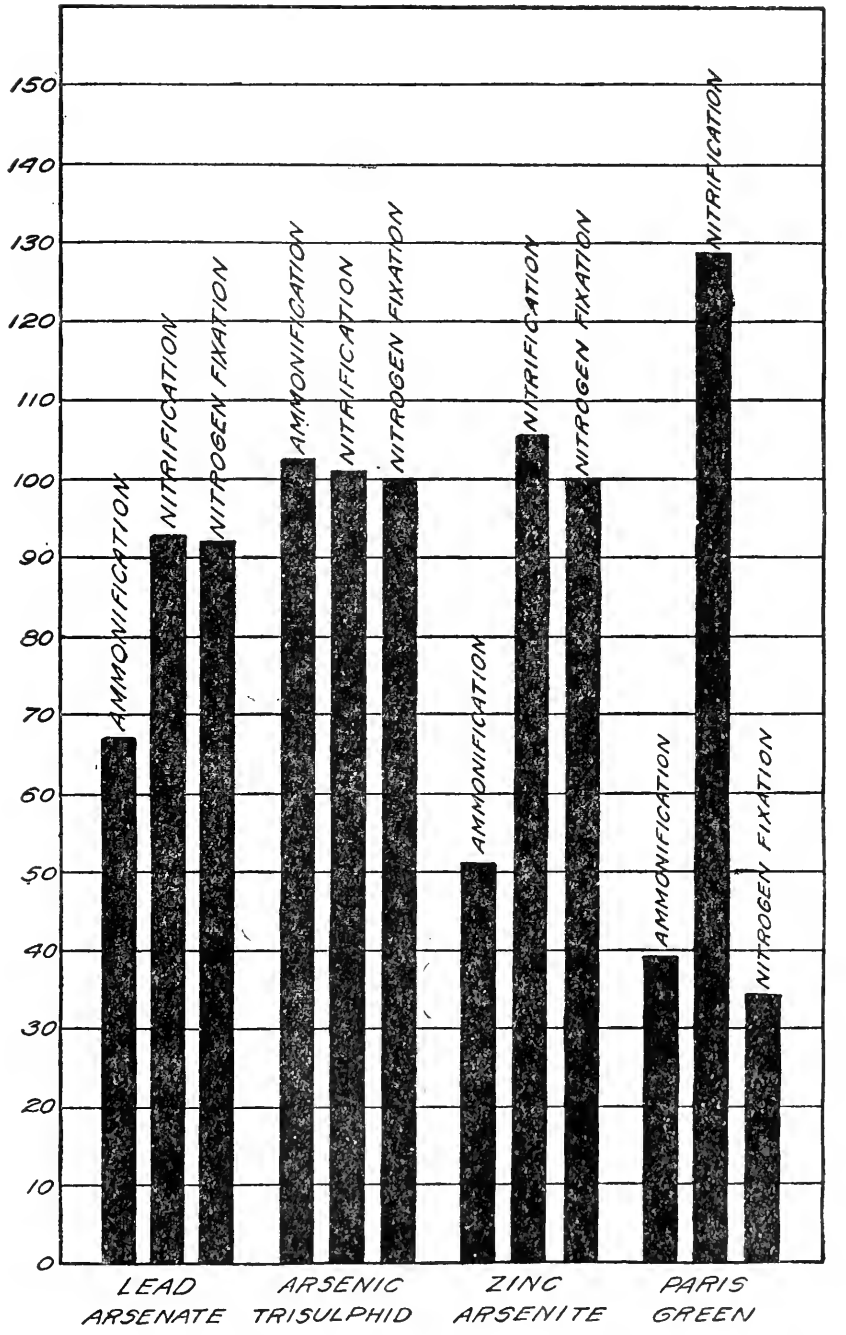


FIG. 4.—Effect of various arsenic compounds in the ratio of 400 parts of the compound to 1,000,000 parts of soil on the activity of various soil organisms.

organism. This set of organisms are, however, more resistant to copper than are others, and what we have occurring is a suppression of other types which feed on nitrates, thus permitting a greater accumulation

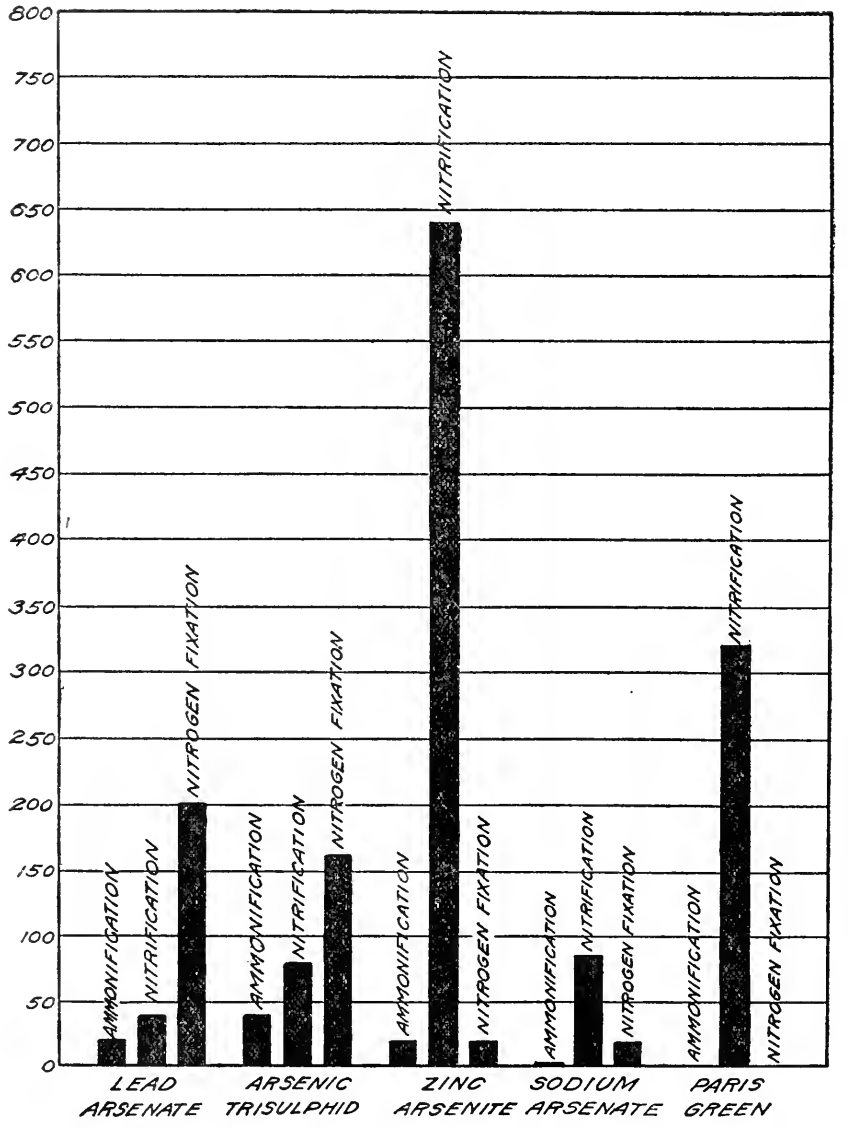


FIG. 5.—Graph showing parts per million of various arsenic compounds in the soil at which the greatest stimulation occurred.

of nitrates under these conditions. While not so likely in the other cases, the same possibility does arise. This, however, can be answered definitely only by further experiments.

SUMMARY

Arsenic, when applied to a soil in the form of lead arsenate, sodium arsenate, arsenic trisulphid, or zinc arsenite, stimulates the nitrogen-fixing powers of the soil. This stimulation is greatest when lead arsenate is applied and least when zinc arsenite is applied. Paris green did not stimulate in any of the concentrations. This compound becomes very toxic when the concentration reaches 120 p. p. m. The toxicity of this compound is due to the copper and not to the arsenic contained in it. Sodium arsenate became toxic when a concentration of 40 p. p. m. of arsenic was added, and when 250 p. p. m. were added it entirely stopped nitrogen fixation. Lead arsenate was not toxic even at a concentration of 400 p. p. m. of arsenic. The toxicity of arsenic trisulphid and zinc arsenite was very small at this concentration.

The stimulation noted when arsenic is added to a soil is not due to any inherent peculiarity of the soil used, for soils which vary greatly in physical and chemical properties had their nitrogen-fixing powers greatly increased when arsenic was applied to them. Soils high in organic matter fixed as much nitrogen in the presence of arsenic and in the absence of mannite as they did in the presence of mannite and absence of arsenic. The stimulation is greatest when the water-soluble arsenic content of the soil is about 10 p. p. m. This quantity exceeds that found in most soils, so it is likely that in agricultural practice arsenic will stimulate and not retard bacterial activity in the soil.

Only one type of Azotobacter was isolated which was stimulated by arsenic, and in this case the stimulation was due to the organism utilizing more economically in the presence of arsenic its source of carbon than it did in the absence of arsenic. The arsenic compounds do not act as a source of energy to the organisms. The main part of the stimulation noted in the soil with its mixed flora is undoubtedly due to the arsenic inhibiting injurious species.

A quantity of arsenic which acts as a stimulant to bacteria when placed in soil may become very toxic when tested by the Remy-solution method.

Arsenic can not replace phosphorus in the vital process of the nitrogen-fixing organisms, but it can in some manner liberate the phosphorus from its insoluble compounds. This may be either a direct or an indirect action.

Arsenic stimulates the cellulose ferments, and these in turn react upon the activity of the nitrogen-fixing organisms.

The nitrogen-fixing powers of soil extract, of filtered soil extract, and soil dried for some time are only slightly stimulated by arsenic, showing that arsenic acts mainly by the removal of a thermolabile body which occurs in the soil.

LITERATURE CITED

ASHBY, S. F.
1907. Some observations on "nitrification." *In* Jour. Agr. Sci., v. 2, pt. 1, p. 52–67.
BRENCHLEY, Winifred E.
1914. Inorganic Plant Poisons and Stimulants. 110 p., 19 fig. Cambridge.
CLARK, H. W., and GAGE, S. DeM.
1906. On the bactericidal action of copper. *In* Jour. Infect. Diseases, Sup. 2, p. 175–204.
CUNNINGHAM, Andrew, and LÖHNIS, Felix.
1914. Studies on soil protozoa. I. The growth of protozoa on various media and the effect of heat on active and encysted forms. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 39, No. 23/25, p. 596–610.
DARBISHIRE, F. V., and RUSSELL, E. J.
1907. Oxidation in soils, and its relation to productiveness. II. The influence of partial sterilisation. *In* Jour. Agr. Sci., v. 2, pt. 3, p. 305–326, 3 fig.
DEMOLON, A.
1913. Recherches sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. [Paris], t. 156, no. 9, p. 725–728.
EHRENBERG, Paul.
1910. Wirkung des Zinks bei Vegetationsversuchen. Zugleich Beiträge zur Ammoniakfrage II. *In* Landw. Vers. Stat., Bd. 72, Heft 1/2, p. 15–142, pl. 1–6.
FRED, E. B.
1911. Über die Beschleunigung der Lebenstätigkeit höhrer und niederer Pflanzen durch kleine Giftmengen. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 31, No. 5/10, p. 185–245, 1 fig.
GREAVES, J. E.
1910. Effects of soluble salts on insoluble phosphates. *In* Jour. Biol. Chem., v. 7, no. 4, p. 287–307.

———
1913a. The influence of arsenic upon the biological transformation of nitrogen in soils. *In* Biochem. Bul., v. 3, no. 9, p. 2–16.

———
1913b. The occurrence of arsenic in soils. *In* Biochem. Bul., v. 2, no. 8, p. 519–523.

———
1913c. Some factors influencing ammonification and nitrification in soils. I. Influence of arsenic. *In* Centbl. Bakt. [etc.], Bd. 39, No. 20/22, p. 542–560.

———
1913d. Some factors influencing the quantitative determination of arsenic in soils. *In* Jour. Amer. Chem. Soc., v. 35, no. 2, p. 150–156, 1 fig.

———
1914. A study of the bacterial activities of virgin and cultivated soils. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 41, No. 11/17, p. 444–459.
GÜNTHER, E.
1897. Beitrag zur mineralischen Nahrung der Pilze. Inaugural-Dissertation. Erlangen. (Abstract.) *In* Bot. Ztg., Abt. 2, Jahrg. 55, No. 24, p. 379.
HARDEN, Arthur.
1911. Alcoholic fermentation. 128 p., 8 fig. London, New York. Bibliography, p. 115–126.
——— and YOUNG, W. J.
1906. Influence of sodium arsenate on the fermentation of glucose by yeast-juice. (Preliminary notice.) *In* Proc. Chem. Soc. [London], v. 22, no. 315, p. 283–284.

HOFFMANN, C., and HAMMER, B. W.
1910. Some factors concerned in the fixation of nitrogen by Azotobacter. Wis. Agr. Exp. Sta. Research Bul. 12, p. 155–172, 2 fig.
JACKSON, D. D.
1905. Purification of water by copper sulphate. (Abstract.) *In* Rev. Amer. Chem. Research, v. 11, no. 12, p. 675 (Jour. Amer. Chem. Soc., v. 27, no. 12). 1905. Original article in Municipal Engin., v. 29, no. 4, p. 245–246. 1905. Not seen.
JÖNSSON, B.
1896. On the influence of arsenic on the germination of seeds. (Abstract.) *In* Exp. Sta. Rec., v. 8, no. 3, p. 232–233. 1896. Original article in Kgl. Landt. Akad. Handl., bd. 35, p. 95–112. 1896. Not seen.
KANDA, Masayasu.
1904. Studien über die Reizwirkung einiger Metallsalze auf das Wachstum höherer Pflanzen. Jour. Col. Sci. Imp. Univ. Tokyo, v. 19, art. 13, 39 p., 1 pl.
KELLERMAN, K. F., and BECKWITH, T. D.
1907. The effect of copper upon water bacteria. *In* U. S. Dept. Agr. Bur. Plant Indus. Bul. 100, p. 57–71.
——— McBETH, I. G., SCALES, F. M., and SMITH, N. R.
1913. Identification and classification of cellulose-dissolving bacteria. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 39, No. 20/22, p. 502–522, 2 pl.
KOPELOFF, Nicholas, LINT, H. C., and COLEMAN, D. A.
1915. Separation of soil protozoa. *In* Jour. Agr. Research, v. 5, no. 3, p. 137–140. Literature cited, p. 139–140.
LAFAR, Franz.
1911. Technical Mycology. v. 1. London. Cites Molisch, p. 37.
LATHAM, M. E.
1909. Nitrogen assimilation of Sterigmatocystis nigra and the effect of chemical stimulation. *In* Bul. Torrey Bot. Club, v. 36, no. 5, p. 235–244.
LIPMAN, C. B., and BURGESS, P. S.
1914. The effect of copper, zinc, iron, and lead salts on ammonification and nitrification in soils. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 6, p. 127–139.
——— and SHARP, L. T.
1912. Toxic effects of "alkali salts" in soils on soil bacteria. III. Nitrogen fixation. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 35, no. 25, p. 647–655, 1 fig.
——— and WILSON, F. H.
1913. Toxic inorganic salts and acids as affecting plant growth. (Preliminary communication.) *In* Bot. Gaz., v. 55, no. 6, p. 409–420.
McGEORGE, W. T.
1915. Fate and effect of arsenic applied as a spray for weeds. *In* Jour. Agr. Research, v. 5, no. 11, p. 459–463.
MILLER, N. H. J.
1914. Agricultural chemistry and vegetable physiology. *In* Ann. Rpts. Prog. Chem., v. 10, 1913, p. 211–232.
MONTEMARTINI, Luigi.
1911. The stimulating effect of sulphate of manganese and sulphate of copper on plants. (Abstract.) *In* Internat. Inst. Agr. [Rome], Bul. Bur. Agr. Intel. and Plant Diseases, year 2, No. 11/12, p. 2467. 1911. Original article in Staz. Sper. Agr. Ital. v. 44, fasc. 7, p. 564–571. 1911. Not seen.
NOBBE, F., BAESSLER, P., and WILL, H.
1884. Untersuchung über die Giftwirkung des Arsen, Blei, und Zink in pflanzlichen Organismus. *In* Landw. Vers. Stat., Bd. 30, p. 381–419, pl. 2.

RIVIÈRE, Gustave, and BAILHACHE, Gabriel.
1913. De l'influence des substances catalytiques. *In* Jour. Soc. Nat. Hort. France, s. 4, t. 14, p. 782–788.
RUSSELL, E. J.
1905. Oxidation in soils and its connection with fertility. *In* Jour. Agr. Sci., v. 1, pt. 3, p. 261–279, 2 fig.

———
1912. Soil Conditions and Plant Growth. 168 p. London, New York. A selected bibliography, p. 154–156.
——— and HUTCHINSON, H. B.
1913. The effect of partial sterilization of soils on the production of plant food. II. The limitation of bacterial numbers in normal soils and its consequences. *In* Jour. Agr. Sci., v. 5, pt. 2, p. 152–221, 7 fig.
SHEDD, O. M.
1914. The relation of sulfur to soil fertility. Ky. Agr. Exp. Sta. Bul. 188, p. 593–630.
SILBERBERG, Berenice.
1909. Stimulation of storage tissues of higher plants by zinc sulphate. *In* Bul. Torrey Bot. Club, v. 36, no. 9, p. 489–500, 4 fig.
STOKLASA, Jules.
1897. De la substitution de l'acide arsénique a l'acide phosphorique dans la nutrition de la plante. *In* Ann. Agron., t. 23, no. 10, p. 471–477.

———
1911. Catalytic fertilizers for sugar beets. (Abstract.) *In* Exp. Sta. Rec., v. 26, p. 225, 1912; Chem. Ztg., Bd. 35, No. 86, Repert., p. 361, 1911. Original article in Bl. Zuckerrübenbau, Bd. 18, No. 11, p. 193–197, 1911. Not seen.

———
1913. De l'influence de l'uranium et du plomb sur la végétation. *In* Compt. Rend. Acad. Sci. [Paris], t. 156, no. 2, p. 153–155.
VOELCKER, J. A.
1913. Pot-culture experiments, 1913. *In* Jour. Roy. Agr. Soc. England, v. 74, p. 411–422, 10 pl.
VOGEL, J.
1914. Die Einwirkung von Schwefel auf die bakteriellen Leistungen des Bodens. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, no. 1/8, p. 60–83.

TRANSMISSION AND CONTROL OF BACTERIAL WILT OF CUCURBITS[1]

By FREDERICK V. RAND, *Assistant Pathologist*, and ELLA M. A. ENLOWS, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

WILT TRANSMISSION

That the striped cucumber beetle (*Diabrotica vittata* Fab.) is a direct carrier of the bacterial-wilt organism (*Bacillus tracheiphilus*) from infected to healthy cucurbits was shown several years ago by Smith.[2] He also expressed the conviction that it was the most important, if not the only, summer carrier, and stated the possibility of its serving also as the winter carrier of the disease. Observation and experiment by the senior writer[3] during the last two seasons have abundantly confirmed the implication of the striped cucumber beetle as a summer carrier and have brought out strong proof that this insect is not only the principal summer carrier but also the winter carrier of the wilt organism. The twelve-spotted cucumber beetle (*D. duodecimpunctata* L.) must be included with the striped cucumber beetle at least as an important summer carrier of the disease.

INSECT TRANSMISSION

Relative to cucumber beetles as winter carriers, several direct cold-storage tests have been carried out by the writers in Washington. During the summer and fall of 1915 hundreds of beetles were collected and placed in cold storage at temperatures ranging from 4° to 10° C. These early experiments were conducted partly with a view to determining the proper conditions of feeding prior to storage and the temperature and humidity most favorable to hibernation in storage. The optimum environment for hibernation varies for different insects, and it is necessary to work out this problem for each species. Consequently in these preliminary tests the greater portion of the beetles placed in cold storage was lost. Infection experiments with the few surviving beetles gave the results here detailed.

EXPERIMENT 1.—Several striped cucumber beetles were collected in October, 1914, and fed about two weeks on cucumber vines (*Cucumis sativus*) wilting as a result of natural infection with *B. tracheiphilus*. After six weeks' hibernation in cold storage the five surviving beetles were caged with a young squash plant on which

[1] Some of the details of the field experiments at East Marion, N. Y., were carried out by Mr. Wayland C. Brown, of the Bureau of Plant Industry. The land used in these experiments was furnished by Messrs. J. H. Douglass and G. S. Nowell, of East Marion.

[2] Smith, Erwin F. Bacteria in relation to plant diseases. v. 2, p. 215. Washington, D. C., 1911.
—— A conspectus of bacterial diseases of plants. *In* Ann. Mo. Bot. Gard., v. 2, no. 1/2, p. 390. 1915.

[3] Rand, F. V. Dissemination of bacterial wilt of cucurbits. *In* Jour. Agr. Research, v. 5, no. 6, p. 257–260, pl. 24. 1915.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
eb

Vol. VI, No. 11
June 12, 1916
G—83

they were allowed to feed for 11 days. Observation after two weeks showed unmistakable signs of incipient wilt around some of the beetle injuries on the leaves—that is, a lighter dull green and slight flaccidity of the tissues. With the expectation that the wilt would extend throughout the leaves the pouring of plates was deferred. However, these incipient infection areas dried up without spreading further, and consequently it was impossible to obtain cultures. That *B. tracheiphilus* was present in the wilted vines fed to these beetles was shown by the subsequent inoculation of cucumbers, cantaloupes, and squashes with cultures obtained from these wilted vines (strains R230 and R235). Numerous inoculations with these two strains have shown them to be virulent upon cucumbers and cantaloupes, but inoculations on several varieties of squash have given nothing more than incipient infection.

EXPERIMENT 2.—On October 25, 1915, striped cucumber beetles were collected at Giesboro Point, D. C., in a squash field where bacterial wilt was very prevalent. These beetles were fed for three days on plants which were wilting as a result of inoculation with pure cultures of *B. tracheiphilus*. They were then placed in small boxes provided with screened covers, and held in the ice compartment of a refrigerator at a temperature of about 10° C. for five weeks and four days. At the end of this time (Dec. 6) the beetles were removed and placed in cages containing young cucumber plants. Four to six beetles were placed in each of the six cages used, each cage containing three young plants. After being allowed to feed on these plants for 10 days the beetles were removed and the plants kept in one of the Department greenhouses where there had been no cucurbit wilt since the preceding spring and where no cucurbitaceous insects were present.

On December 17 leaves injured by the beetles on three of these plants were wilted. Microscopic examination showed bacteria present in great number in the vessels of the petioles, and poured plates from the wilted leaves and petioles gave pure cultures of the wilt organism (strain R313). Needle-prick inoculations from these cultures again gave typical wilt on cucumber plants. On December 24 a gnawed leaf on a fourth plant was found wilting, and was removed from the plant. Enormous numbers of bacteria were present in the vascular tissues, and cultures (strains R315 and En126) isolated therefrom gave also successful infection when pricked into the leaves of young cucumber plants. From the portion of petiole remaining the wilt gradually extended throughout the plant, which finally collapsed. On January 4 another plant was found entirely wilted. The gnawed leaf which had wilted first, and from which the wilt had spread throughout the plant, was photographed and preserved. Cultures (strain En124) and paraffin sections (En36) were made from the petiole of this leaf. The organism isolated gave typical infections when inoculated into cucumber plants.

EXPERIMENT 3.—Another lot of *D. vittata* collected in the squash field referred to in experiment 2 was fed for three days on old wilting stems of squash (*C. maxima*) collected in the same field. After keeping these beetles in storage for two months under the same conditions as in experiment 2, they were removed and caged for five days with 12 young cucumber plants. Although these plants were under observation for over two months no wilt appeared in any of them.

EXPERIMENT 4.—On December 16, 1915, five specimens of *D. vittata* and four of *D. duodecimpunctata* hibernating under natural conditions in the squash field at Giesboro Point, D. C., were sifted from the surface soil and taken to the greenhouse. The striped and spotted beetles were placed at once in separate cages, each containing three young cucumber plants. Although the beetles fed freely on these plants, the results of this experiment were negative.

The negative results in experiment 3 possibly may be explained by the fact that the wilted plants fed to the beetles were old, ripe squash

vines which had been diseased for a long time. Doubtless few living organisms were present, since great difficulty was experienced in obtaining cultures of *B. tracheiphilus* from this field (strains En102 and En110). The beetles used in experiment 4 were collected when hibernating in a field where wilt was known to have occurred, but it is evidently not possible to determine whether they had fed upon wilted plants. On the other hand, it is not reasonable to assume that all beetles which have fed upon wilted plants would necessarily be able to carry infection on their mouth parts for any great length of time. Experiments 1 and 2 show that at least in some cases the striped beetles may carry the wilt organism for at least five or six weeks and still be able to infect healthy plants. This, in connection with the field experiments previously published,[1] seems to establish beyond doubt that *D. vittata* is a winter carrier of the cucurbit organism.[2] Experiments with other species of insects have thus far given negative results, as here detailed.

In each of seven tests carried out with the common squash bug (*Anasa tristis* DeG.) during the summer and fall of 1915 in field and greenhouse, two to six of these insects were fed for one to three days on wilted cucumber leaves and petioles and then inclosed with several healthy cucumber plants. After feeding on these plants for one to two days the bugs were removed and the plants kept under observation for three to four weeks. No wilt appeared in any of these plants, but no absolute conclusion can be drawn from the negative results of so small a series of tests.

The twelve-spotted (or squash) lady beetle (*Epilachna borealis* Fab.) was very scarce in eastern Long Island during the season of 1915, but two tests with it similar to those outlined above gave negative results.

The melon aphis (*Aphis gossypii* Glov.) and the flea beetle (*Crepidodera cucumeris*) apparently do not serve as wilt carriers. This has been shown by the negative results from transfer of insects fed upon wilted plants to healthy cucumber plants in insect-proof cages (three tests), and by the fact that no wilt developed during the season in cucumber plants grown in 48 large screened cages (East Marion, Long Island, N. Y., 1915), although numerous wilted plants occurred around all of these cages, and aphids and flea beetles had free access through the meshes of wire netting and were abundant both outside and inside the cages.

In only 2 out of 50 cages did wilt appear and in these cases striped cucumber beetles had gained access or had been purposely introduced, and the disease had started from points gnawed by the beetles.

[1] Rand, F. V. Op. cit.

[2] Wild cucurbits may be eliminated as possible carriers of bacterial wilt so far as the experiments at East Marion are concerned. Personal observations, together with those of Burnham and Latham (Burnham, Stewart H., and Latham, Roy A. The flora of the town of Southold, Long Island and Gardiner's Island. *In* Torreya, vol. 14, nos. 11–12, 1914), and a search through the herbaria of the New York and Brooklyn Botanical Gardens, have established beyond doubt that no wild Cucurbitaceae occur within 10 to 15 miles of the experimental plots.

In each of eight direct summer field tests, one to five striped cucumber beetles were fed for one to three days on wilting cucumber leaves and petioles and then at once caged up with several healthy young cucumber plants. In six out of these eight tests bacterial wilt appeared in one to two weeks and only on plants gnawed by the beetles.

In the two fields (East Marion, Long Island, N. Y.) where spray tests were carried out during the season of 1915 the prevalence of bacterial wilt closely followed that of the striped cucumber beetle. Throughout the season careful and frequent observation failed to disclose a single case of wilt which had not evidently started in a part of the plant injured by cucumber beetles (Pl. LIII). In these two fields no wilt had appeared up to the 1st of July. A few cases were observed on July 3, while the greatest number of cases was found during the last 10 days of the month. Practically no new cases of wilt appeared after the 30th of July. The first striped cucumber beetles of the season were seen from June 15 to 17. In field 1 the first beetles were found on June 17 between cages 14 and 15.[1] On July 3 there were only seven cases of wilt in the whole field, and six of these occurred near or about where these beetles had been collected. The beetles were most numerous between June 24 and July 8, in fact so numerous that in order to save the plants from entire destruction an application of a proprietary dust insecticide (containing lime, Paris green, etc.) was made upon the unsprayed plots. Thus, for a few days, or until new growth appeared on the vines, there were no untreated cucumber plants in these two fields upon which the beetles could feed. From this date on, the beetles began to disappear from these fields. In the variety-test block and commercial fields in the vicinity the plants were younger and for the most part were untreated. In fact, most commercial plantings were just breaking through the ground on July 10. Such fields present an attractive feeding ground for the beetles. In the two experimental fields there were only a few beetles present on July 15, and they were exceedingly scarce after July 30.

When it is remembered that under field conditions usually one to three weeks elapse between time of infection and the appearance of wilting in the plants, it will be seen that the rise and fall in the number of plants with bacterial wilt closely follows the rise and fall in the number of beetles (fig. 1).

The two fields just discussed had been planted to cucumbers the preceding season. About a quarter of a mile from field 1 a cucurbit variety test block was located. This land had not been plowed for several years. Although separated only by slightly rolling, plowed land from field 1, where striped cucumber beetles appeared on June 17, no beetles appeared here until about the end of the first week in July. This was just after

[1] These beetles were used in the cage transmission tests recorded in a former paper (Rand, F. V., op. cit.) and mentioned in a preceding paragraph.

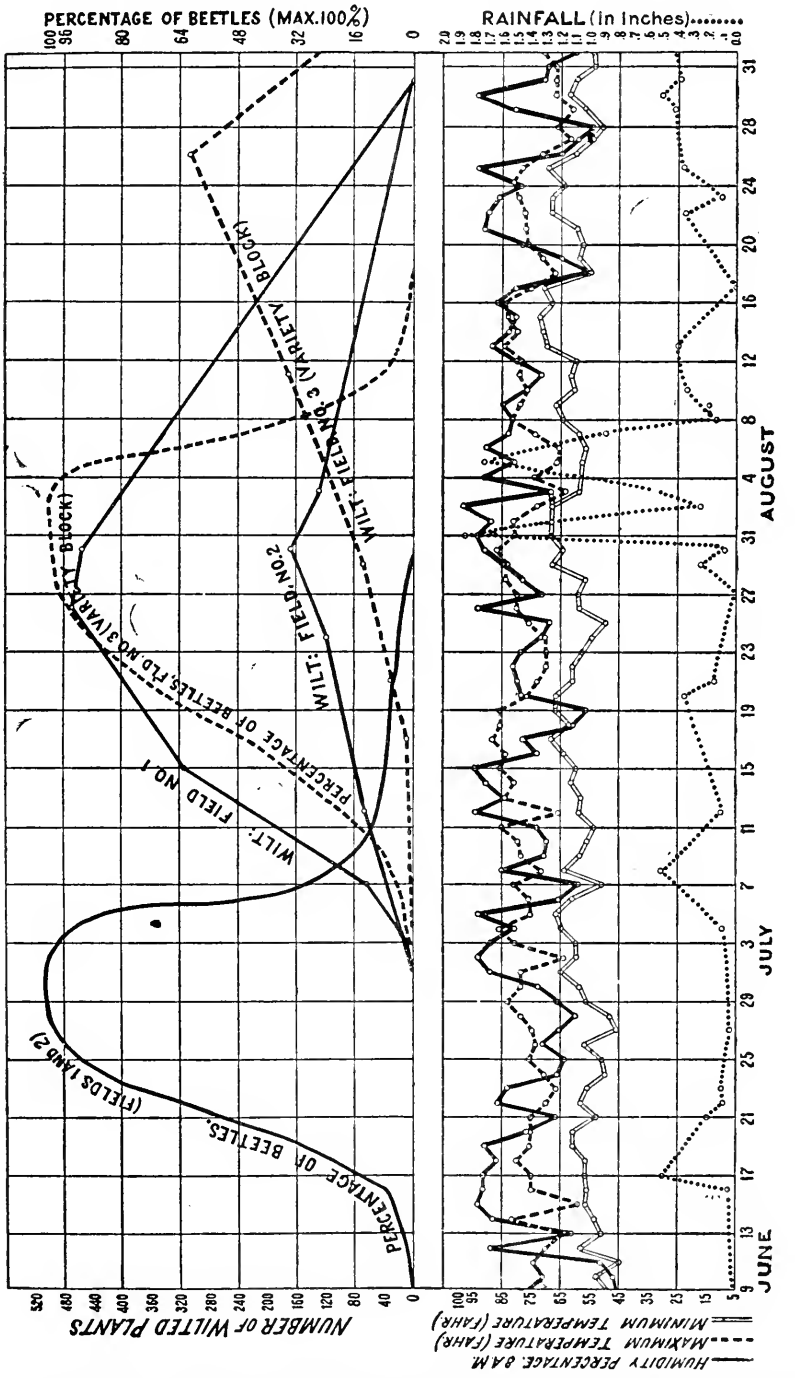


FIG. 1.—Comparison of the amount of wilt with striped-beetle prevalence and with meteorological phenomena in three fields, East Marion, Long Island, N. Y., season of 1915.

they had begun to disappear from field 1. In the variety test the first scattered cases of wilt were observed on July 17, whereas in field 1 the first cases were noted on July 3. The largest number of cases of wilt in the variety block were found between August 15 and 30, and the striped cucumber beetles were most numerous here during the last part of July. Again, allowing for the necessary time between infection and actual wilting, it will be noted that here also there is a direct relation between the number of wilt infections and the number of beetles present (fig. 1).

The graphs (fig. 1) show the daily relation between meteorological conditions, the number of beetles present, and the number of wilted plants in the three fields from June 10 to August 31. In these graphs there is shown a definite relation between the beetle and the wilt curves, but no relation between the latter and the meteorological curves. The meteorological instruments from which the data were obtained for this graph were kept in a United States Weather Bureau instrument shelter at ground level, so that the environment would correspond as nearly as possible to that of the cucumber plants (Pl. LIV, fig. 4).

Reference should be made to the fact that in taking notes the total number of plants showing bacterial wilt was recorded at each date of observation. This number included not only the new cases but also cases holding over from the preceding observation. Ordinarily the older the plant at the time of infection the longer the interval between infection and death. This explains the apparently too great interval between the maxima of the beetle and wilt curves. If it had been the original intention to represent graphically the relation between the prevalence of the beetles and the occurrence of wilt, the data would have been obtained in a form better suited to this method. It was only after tabulating the results of the field observations that the very striking parallel was noted. Obviously it would be impossible to enumerate absolutely the beetles present in a field; hence, the percentages used in the graphs are based partly on actual counts and partly on careful estimates made throughout the season. In the curves, 100 per cent represents the maximum number of striped cucumber beetles present at any one time.

Attention should be drawn to the fact that although there was a difference of only three days in planting time between field 1 and field 3, the beetles appeared between two and three weeks earlier in field 1, which had been planted to cucumbers the preceding season. This would suggest that these insects hibernated in or near the old cucumber field and that they did not leave this field the following spring as long as young and tender plants remained for them to feed upon. A similar tendency of both striped and twelve-spotted cucumber beetles to hibernate in old cucurbit fields was observed by the writers near Congress Heights, D. C. The first frosts occurred in these fields during the first part of October. About the middle of December, 1915, soil siftings to

a depth of 7 inches were made at numerous points over this squash field. Considerable numbers of dormant beetles were found under clods, old vines, mummied squashes, and around the bases of old squash stems just beneath the soil surface. No beetles were found below the first 2 inches and most of them were found at a depth of less than 1 inch.

SOIL TRANSMISSION

In the experiments of 1915 at East Marion, Long Island, N. Y.,[1] bacterial wilt was not transmitted to the plants from the soil, although in the same fields during the preceding season the crop had been largely destroyed by this disease. In a large number of greenhouse inoculations into one of two or more cucurbit plants in a single pot (seven experiments, including in all 126 pots), none but the inoculated plants ever took the disease, although the latter wilted to the ground, and the pots were kept under observation from one to three months. The house was free from cucurbitaceous insects.

In addition to these observations and experiments relative to soil transmission three series of direct soil inoculations were made:

SERIES OF MARCH 18, 1915.—Thirty-two Arlington White Spine forcing cucumber plants 4 to 5 inches high, transplanted March 9 and not disturbed from that date until the date of inoculation, were inoculated as follows:

Eight cucumber plants not root-pruned and the same number of plants root-pruned were inoculated with strain R230 by pouring on the soil beef-bouillon cultures 6 days old. Sixteen plants were inoculated in the same way with strain R235. Sixteen plants were root-pruned and the soil moistened with tap water only, these plants being held as checks. The cultures used were tested as to virulence by needle-puncture inoculations into the leaves of several cucumber plants of the same age and variety.

All plants inoculated by needle puncture promptly wilted.

On April 1 the 16 inoculated plants which had been root-pruned showed two cases of wilt. No wilt was evident in the 16 non-root-pruned plants at this date.

On April 12, among the 16 root-pruned plants there were 10 wilted and among the 16 non-root-pruned there were 2 wilted. The 16 check plants (root-pruned) showed no signs of wilt.

Isolations were made from all plants showing infection from the soil, and these cultures produced wilt promptly upon inoculation into leaves of healthy plants.

The experiment was continued for two months from the date of inoculation, but no further cases of wilt appeared.

SERIES OF MARCH 31, 1916.—Sixty Chicago Pickling cucumbers planted January 28, 1916 (transplanted once), in pots in the greenhouse

[1] Rand, F. V., 1915. Op. cit.

were inoculated by pouring on the soil tap-water suspensions of *B. tracheiphilus* from beef-agar slants 6 days old. Of these 60 plants 24 were root-pruned, and the remaining 36 were left uninjured. In this experiment 26 strains, isolated from squash, cucumber, and cantaloupe (*Cucumis melo*), were used, and each culture was proved to be virulent by needle-puncture inoculation into the leaves of healthy cucumbers of the same age and variety. The virulence tests were made about 30 minutes before these agar slants were used for the soil inoculations.

The plants were under daily observation, and there were no signs of wilt until April 11, when one of the root-pruned plants wilted. Between this date and April 19, 6 of the 24 root-pruned plants (25 per cent) and 8 of the 36 uninjured plants (22 per cent) wilted. Examination of the stems and main roots showed the typical stringy slime in the vascular system, and cultures from these roots proved the presence of *B. tracheiphilus*.

It will be seen that in this test the percentage of wilt was about the same in the root-pruned plants and in those not root-pruned. However, too much weight can not be given to the results of this experiment, since the cucumbers had been recently transplanted and examination of the roots showed considerable eelworm injury. These wounds might, of course, afford entrance for the wilt organism.

SEED TRANSMISSION

Ripe cucumber fruits were collected from wilted vines at Malone and Constable, N. Y., on September 23, 1914. Five of the fruits from Malone and one from Constable showed on cutting an abundance of the sticky white ooze characteristic of this bacterial wilt, and microscopic examination revealed enormous numbers of typical bacteria in the vascular system. The seeds were carefully preserved, and three months later were planted in the greenhouse. Good germination resulted, and after three months' growth no signs of wilt had occurred in any of the plants.

In July, 1915, a large White Spine cucumber fruit almost full grown was inoculated from a pure culture of the bacterial wilt organism. The fruit became infected and the wilt extended gradually throughout the whole vine to which it was attached. Seeds from this fruit were preserved, and six months later a part of them were planted in the greenhouse. Several plants came up and were under observation for four weeks, but no wilt occurred. A portion of the seeds remaining were used in cultural tests. The seeds were sterilized in the usual way with mercuric-chlorid solution, the seed coats removed under aseptic conditions, and the embryos crushed in sterile bouillon from which plates were poured. No clouding of the bouillon subsequently occurred, and no growth from the plates.

On August 29, 1914, a ripe cantaloupe was collected from a wilted vine near Albany, N. Y. The vascular elements of the cantaloupe con-

tained an abundance of the typical stringy ooze which microscopic examination showed to consist entirely of characteristic bacteria. Seed germination and cultural tests similar to those described for the cucumber gave negative results.

During the latter part of September, 1914, ripe Hubbard squashes were collected from wilted vines at Medina, Malone, and Constable, N. Y. These squashes upon examination showed the same evidence of bacterial wilt as did the cucumbers and cantaloupes referred to above, and in addition a pure-culture isolation of *B. tracheiphilus* was made from the Medina squash, which subsequently gave typical infections when inoculated into healthy cucumber and squash plants. Seed germination and cultural tests from the seeds gave the same negative results as in cucumbers and cantaloupes.

STOMATAL INFECTIONS

Two inoculation tests with cucumber and one with cantaloupe were made during the summer of 1915, using sterile-water suspensions of the wilt organism (strain R230). The plants were put into tight inoculating cages, and the plants and walls of the cages sprayed with tap water. Two hours later the plants were inoculated by spraying with a very cloudy suspension of bacteria from 7-day-old agar slants. Check plants were inoculated by needle punctures from the same cultures. All the plants were left in the cages tightly closed for 24 hours, and semiopen for two days longer. The punctured checks wilted promptly, but no infection occurred in the uninjured sprayed plants, although they were kept under observation for two months. Another test was made in March, 1916. Three young and three older cucumber plants and four young squash plants were inclosed in a dampened inoculation chamber and sprayed with a tap-water suspension of *B. tracheiphilus* (strain En58 isolated from squash) from a 6-day-old beef-agar slant. Three hours later the plants were again sprayed with this bacterial suspension. This culture was at the same time tested by needle-puncture inoculations into the leaves of two cucumber and two squash plants of the same varieties. The sprayed plants were left in the inoculating chamber in a saturated atmosphere for three days, after which they were held under ordinary greenhouse conditions. After two months no infection had appeared in the sprayed uninjured plants, although the plants inoculated from the same culture by needle punctures all developed typical wilt within one week after inoculation.

A fifth trial was made in April, 1916, using five young and five older cucumber plants. All aerial parts were thoroughly sprayed with a tap-water suspension of the wilt organism from a beef-agar slant 6 days old (strain En57). This culture was tested by needle-puncture inoculations into cucumbers of the same age and variety. The latter inoculations resulted in typical wilt, but the uninjured plants sprayed with the bacterial suspension had shown no signs of infection after five weeks.

In these five direct tests stomatal infection did not occur, thus confirming the observational data during the past two seasons and Dr. Smith's earlier observations and experiments. In hundreds of field and greenhouse observations the stems and leaves of wilted and healthy plants were closely intertwined, exposing in many cases uninjured healthy parts to direct contact with cut and broken infected surfaces. Even here the disease was in no case transmitted.

DISCUSSION OF OBSERVATIONS

The field observations of the senior writer during the last two seasons, covering the territory from the District of Columbia to eastern Long Island, northward to the Canadian Provinces of Quebec and Ontario, and westward to Michigan, Wisconsin, and Indiana, have abundantly confirmed the experimental evidence outlined above that the striped cucumber beetle and probably also the twelve-spotted cucumber beetle are the principal if not the only carriers of bacterial wilt of cucurbits. It has been suggested that the larvæ of cucumber beetles may also serve as a means of dissemination, but from their habits it would appear that the only possible way in which they could bring about infection is by carrying the organism from the soil into their burrows in the cucumber stems. This appears highly improbable. However, the data at hand do not warrant any definite statement.

Mechanical injuries, such as those resulting from storms, cultivation, etc., and injuries from flea beetles, aphids, and squash bugs have been closely watched in the experimental fields and cages described elsewhere, but no evidence has been obtained of any relation to bacterial wilt.

WILT CONTROL

The problem of control therefore resolves itself into (1) the finding or developing of cucurbit varieties resistant to bacterial wilt, (2) spraying the plants with a bactericide, or (3) eliminating the beetles through poisons or repellants.

VARIETY TESTS

Early in the spring of 1915 a preliminary test was made with eight varieties of cucumber planted in pots in one of the department greenhouses. Several plants of each variety were inoculated by needle punctures in the leaves from 6-day-old agar-slant cultures of a single strain of *B. tracheiphilus*. All the inoculated plants contracted the disease and no difference in rapidity of wilting appeared—that is, individual plants of the same variety showed as great differences in rate of wilting as appeared among the different varieties.

In the variety-test block previously mentioned (East Marion, Long Island, N. Y.) 32 varieties of cucumber, 39 varieties of cantaloupe, and 25 varieties of squash were planted on June 10, 1915. From 8 to 20 or more hills were given to each variety, 12 being the usual number. Most of the cucumber and squash varieties gave fair to good stands, but the cantaloupes were planted in an exceedingly light sandy soil infested with witch grass, and in consequence of this the seed either did not come

up at all or gave a very poor stand of plants. Only seven of the cantaloupe varieties were in such location and condition as to be included in a summary of results. It was intended at first to inoculate artificially at least one plant of each variety with the wilt organism, in order that all varieties might have an equal chance, but the disease soon became so general over the experimental block that it was thought unnecessary to interfere with its natural spread.

Careful observations were made throughout the season and the number of wilted plants in each variety was noted. Table I gives the percentage of wilted plants for each variety during the season.

TABLE I.—*Percentage of wilt in different varieties of cucumbers, squashes, and cantaloupes at East Marion, Long Island, N. Y., season of 1915*

CUCUMBER

Variety.	Percentage of wilt.	Variety.	Percentage of wilt.
West India Gherkin	30	Improved Long Green	66
Rollistons Telegraph	33	Fordhooks Famous	70
Emerald	33	Vaughans XXX Pickling	71
Cool and Crisp	33	Cumberland Pickling	75
Vaughans Prolific	40	Fordhook Pickling	77
Lemon	40	Early Cyclone	77
Westfield Chicago Pickling	44	Improved White Spine	77
Snows Fancy Pickling	45	Arlington White Spine	80
Davis Perfect (regular stock)	50	Arlington White Spine (U. S. 19300)	83
Davis Perfect (selected stock)	50	Improved Long Green (U. S. 18591)	83
Noas Forcing	50	Early Cluster	83
Extra Early Long White Spine	66	Serpent or Snake	88
Boston Forcing White Spine	66	Carters Model	90
Improved Jersey Pickling	66	New Century	100
Boston Pickling (U. S. 18589)	66	Grand Rapids Forcing	100
Rockyford Klondyke	66		
Japanese Climbing	66		

SQUASH

Mammoth White Bush	0	Improved Hubbard	42
Early White Bush (U. S. 19339)	0	Pikes Peak	42
Vaughans Giant Summer Crookneck	10	Delicata	50
Early White Bush	12	Essex Hybrid	50
Early White Bush (U. S. 19340)	12	Delicious	60
Mammoth Yellow	14	Faxons Brazilian	70
Giant White Summer	16	Chicago Market Hubbard	75
Straight Neck	20	Orange Marrow	87
Bush Fordhook	26	Red or Gold Hubbard	88
Fordhook	30	Marblehead	100
Yellow Bush	40	Golden Bronze	100
Summer Crookneck	40	Boston Marrow	100
		Vegetable Marrow	100

CANTALOUPE

Emerald Green (U. S. 19352)	9	Burrell's Gem (U. S. 19312)	25
Landreths Early Citron	12	Burrell's Gem (U. S. 19348)	25
Rockyford (U. S. 19319)	15	Vegetable Peach	28
Rockyford (regular stock)	15	Oval Netted Gem	66
Netted Gem Rockyford (selected stock)	18		

None of the 30 varieties of cucumber were free from wilt, the diseased plants in each variety ranging from 30 to 100 per cent. In the 7 varieties of cantaloupe exposed to infection, the wilt ranged from 9 to 66 per cent. Of the 24 varieties of squash, 2 remained free from wilt throughout the season, and in the remaining 22 varieties the disease occurred in 10 to 100 per cent of the plants. Little hope of finding a high degree of resistance is to be noted in the cucumber record. A considerable difference in percentages of wilt is found, but whether this will persist from year to year or is merely accidental can be ascertained only by further trials in different localities and seasons. A greater promise of resistance was evidenced by the squash varieties. In his experiments Dr. Erwin F. Smith, Bureau of Plant Industry, obtained infection in squashes with *B. tracheiphilus* obtained from wilted squash plants, but with cultures obtained from cucumbers squash infections were rare or where they did occur failed to extend beyond the immediate vicinity of inoculation.

Experiments relative to the infection of squash plants by means of cultures of *B. tracheiphilus* obtained from cucumbers, cantaloupes, and squashes are not yet completed. However, up to the present time, 15 strains from cucumber, 1 from cantaloupe, and 7 from squash have been tested by inoculation into these three hosts. All the strains have proved infectious on cucumber and cantaloupe. Of the 15 cucumber strains inoculated into the Yellow Crookneck and Early White Bush squashes, 7 have given no infection, 2 (En66 and En68) have given doubtful signs of incipient wilt, 4 (En68, En109, R305, and R307) primary wilt (not extending beyond the inoculated leaves), and 2 (R308 and En108) wilt involving the entire plant. The single cantaloupe strain in most cases failed to infect squash. In those cases where infection did occur, the signs did not extend far beyond the inoculation punctures. All the squash strains were infectious to squash, varietal differences, however, being evident.

Among the common cultivated cucurbits cucumbers appear to be the most susceptible, and following them in succession should be placed cantaloupes, squashes, and pumpkins, with watermelons (*Citrullus vulgaris*) as most resistant. So far as the writers know, bacterial wilt has been reported but once as occurring naturally upon watermelons, and this case was reported without accompanying proof.[1] The ordinary watermelon wilt is caused by a species of Fusarium.

Summarizing the season's work upon cucurbit varieties, together with the general field observations of the senior writer, it may be stated that there is little hope of controlling the disease in the cucumber through host resistance to the parasite. The cantaloupe and squash, especially

[1] Selby, A. D. Certain troublesome diseases of tomatoes and cucurbits. *In* Ann. Rpt. Columbus Hort. Soc. 1896, p. 113. 1897.

the latter, showed a considerably greater evidence of resistance. For these plants, therefore, this method of control is at least worthy of further investigation, but up to the present time the observations and experiments do not justify definite conclusions.

SPRAYING

In two fields situated near the variety-test block a series of spraying experiments was carried out in 1915 upon the Fordhook Famous cucumber, planted on June 5 and 7, and Woodruffs Hybrid cucumber, planted on June 1. The relation between the striped cucumber beetle and wilt in these two fields has already been detailed (p. 420 and fig. 1). The relative merits of Bordeaux mixture alone, Bordeaux mixture with lead arsenate, and lead arsenate alone were tried out by spraying different plots with each of these three mixtures on a succession of dates, beginning June 25 and continuing at intervals of 5 to 10 days thereafter (fig. 2).

To determine the best time for treatment, the first application of the Bordeaux-mixture-lead-arsenate combination was made upon different plots at successive dates. The first application was made on June 25, just as the first true leaves had opened on the cucumber plants, and at each succeeding application a new plot was added. In every case a check plot was left between the two successively sprayed plots. In field 2 each plot consisted of three parallel rows, each row 21 feet long. In field 1 the plots were about twice this size. The first three applications of Bordeaux mixture were made with a weak suspension (2:2:50) in order not to injure the young plants, but in the later treatments the strength was gradually increased to the 4:6:50 formula. In all cases where lead arsenate was used it was applied at the rate of 2 pounds to 50 gallons of liquid. No appreciable injury from any of the spray mixtures was observed.

The relative amount of control effected in field 1 at different dates of application is graphically shown in figure 3.

The spray treatments were conducted as follows: Plot 1 (fig. 3a) received its first application of Bordeaux and lead arsenate on June 25, and additional sprayings at intervals of 5 to 10 days throughout the season. In plots 2, 3, and 4 (fig. 3, b, c, and d), the first applications were made on July 6, 14, and 19, respectively, and further sprayings were made at intervals of 5 to 10 days as in plot 1. It will be noted that most of the infections had occurred before the third treatment, July 14, for in plot 3 and its corresponding check the number of wilted plants was about the same. In the first two plots there was much less wilt than in the corresponding unsprayed plots, the first sprayed plot showing by far the best results. There would be a still greater difference between plot 1 and its untreated check were it not for the facts that the latter was only about three-fourths the size of the sprayed plot and that it received one applica-

tion of a dust insecticide to prevent the total destruction of the plants by striped cucumber beetles.

The relative amount of control given by the three kinds of spray mixture tested is shown in figure 2, in which the number of wilted plants in each sprayed and check plot is given. It will be noted that the lead arse-

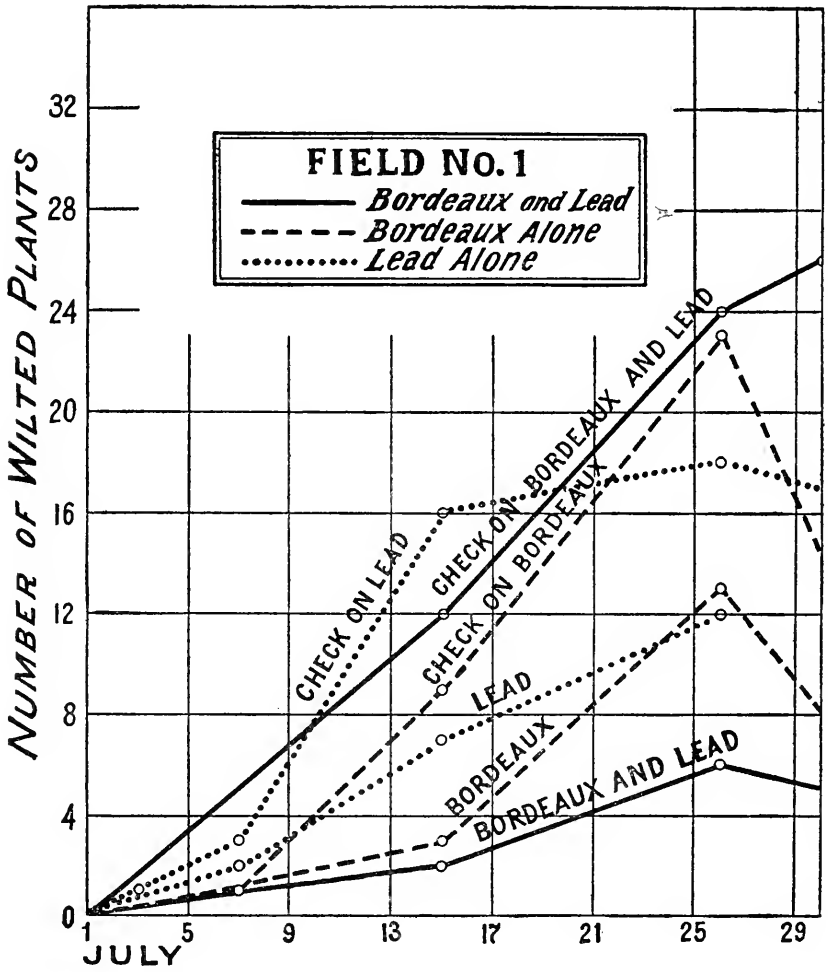


FIG. 2.—Comparison of relative wilt control of Bordeaux mixture plus lead arsenate, Bordeaux mixture alone, and lead arsenate alone in field 1, East Marion, Long Island, season of 1915.

nate and Bordeaux mixture combined gave better results than either used alone.

The results obtained in field 2 are corroborative of the data graphically shown for field 1 (fig. 2 and 3), but the control effected was not quite so striking, since the plants were nearly a week older than those in field 1 at the time of the first spraying. Furthermore, the stand was poor in some

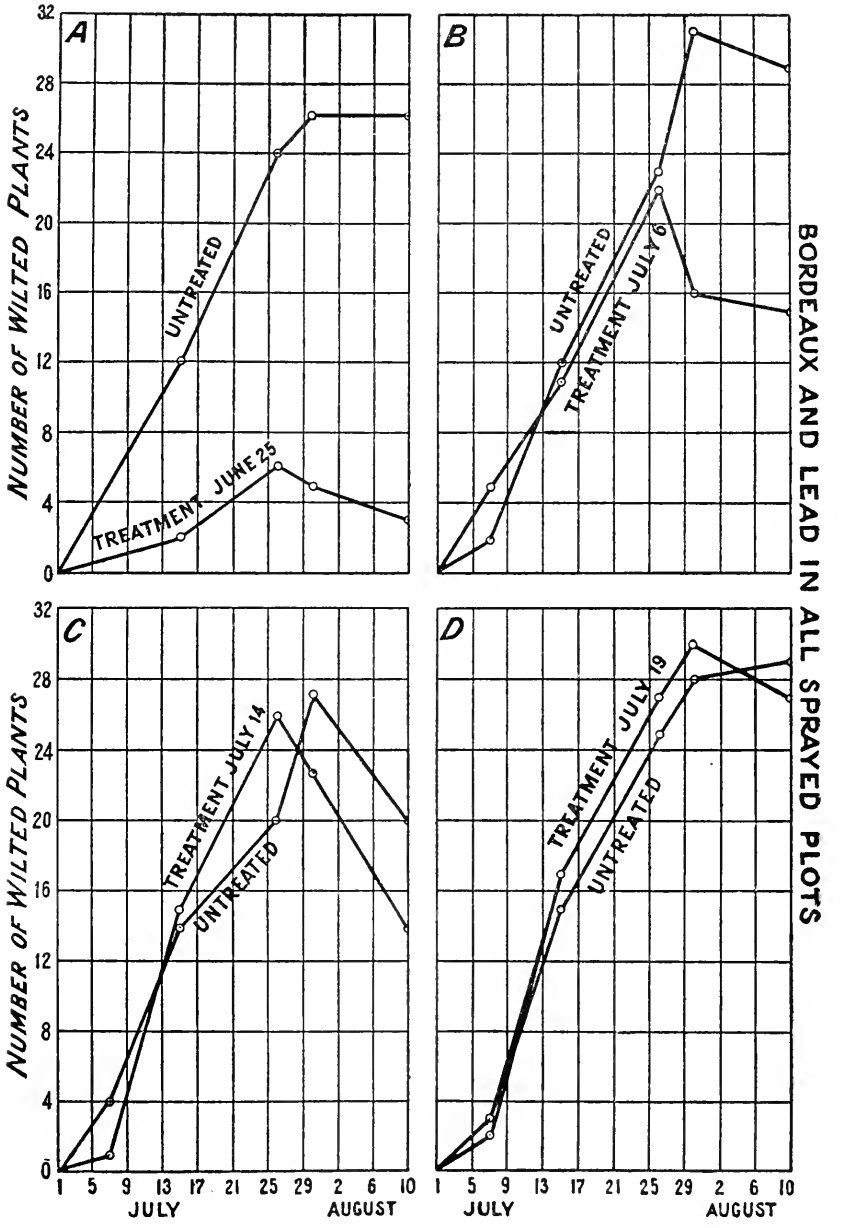


FIG. 3.—Curves showing relative wilt control of Bordeaux mixture and lead arsenate with date of first application as a variant in field 1, East Marion, Long Island, season of 1915.

37768°—16——4

parts of the field and the beetles appeared here a few days earlier than in field 1.

Field observations indicate that the results obtained were due to a bactericidal and repellent action of the Bordeaux mixture and lead-arsenate combination, and probably in part to an insecticidal action by the latter ingredient. The beetles were less frequent on the sprayed than on the unsprayed plots, and among the sprayed plants injured by beetles there was apparently a smaller percentage of infection resulting than among similar unsprayed plants. That is, the control effected by the Bordeaux mixture alone was apparently due to its repellent and bactericidal action, and that by the lead arsenate alone to its repellent and insecticidal action, while the more complete control by the two mixtures together was due to a combination of their bactericidal, repellent, and insecticidal properties.

The bactericidal action of Bordeaux mixture has been further investigated in a series of six greenhouse tests, in which sprayed and unsprayed leaves of potted plants were inoculated in as nearly an identical manner as possible by needle punctures from cultures of the same strain of organism. The spray used was 2:3:50 Bordeaux, and this was allowed to dry thoroughly on the leaves before inoculating. In most cases the plants were not inoculated until 24 hours after spraying.

In the first test, December 2, 1915, three weeks after planting, seven unsprayed and seven sprayed Chicago Pickling cucumber plants were inoculated from 1-week-old beef-agar slant cultures. After 15 days the unsprayed plants showed 100 per cent of infection, and the sprayed plants 29 per cent.

In the same way and at the same time a test was carried out on three varieties of cantaloupe—Rockyford, Sweet Air, and Baltimore Nutmeg. Thirty-five inoculations were made into unsprayed plants and 37 into sprayed plants. There was no apparent difference in susceptibility among the three varieties used. Of these inoculations the unsprayed gave 95 per cent of infection and the sprayed leaves 46 per cent.

In a third test (Jan. 8, 1916), Chicago Pickling cucumbers planted November 13, 1915, were used. In this test 36 unsprayed and 37 sprayed plants were inoculated with the wilt organism, using agar slants 9 days old. After 19 days it was found that 92 per cent of the unsprayed and 35 per cent of the sprayed cucumbers had contracted the disease.

A further trial was made (Jan. 19, 1916) with 19 Chicago Pickling cucumbers planted October 29, 1915. In the case of these older cucumbers unsprayed and sprayed leaves on the same plant and as nearly of the same age and appearance as possible were used for inoculation. Both sprayed and unsprayed leaves had been dusted with flowers of sulphur for the control of powdery mildew, and this treatment, together with the age of the plants, considerably reduced the infection. However, even here the unsprayed leaves gave 63 per cent and the sprayed leaves 11 per cent of infection.

Two more tests (Jan. 19, 1916) were made with Baltimore Nutmeg cantaloupes planted November 13, 1915, using a bacterial strain of low virulence (strain R311) and one of high virulence (strain R304). The cultures of these two strains used for inoculation were beef-agar slants 10 days old. With the former strain 10 unsprayed and 9 sprayed plants were inoculated, and these gave, respectively, 40 and 11 per cent of infection. With the highly virulent strain 16 unsprayed and 17 sprayed plants were inoculated. These gave, respectively, 94 and 24 per cent infection.

Remarks: It will be seen that in all cases the presence of Bordeaux mixture on the leaves greatly reduced infection, and an average of the six trials gives 80.6 per cent of infection in the unsprayed against 26 per cent of infection in the sprayed plants. These results can scarcely be considered as accidental, and they strongly confirm field observations regarding the bactericidal effect of Bordeaux mixture. Furthermore, the natural mode of inoculation is considered identical with the method used in these tests, for in the one case the organism is pricked into the leaf tissues by the mouth parts of the cucumber beetle and in the other case by the inoculating needle.

Wet and Dry Inoculations

On January 8, 1915, an experiment was conducted to determine the effect of wet and dry inoculations into sprayed and unsprayed plants. In this test 68 cucumber plants were used. The inoculations were all made in a uniform manner by needle punctures into the two youngest, fully opened leaves of each plant. Of these plants 34 were sprayed with Bordeaux mixture and 17 were inoculated before the Bordeaux mixture had dried. The remaining 17 were inoculated about 2 hours later when the Bordeaux mixture was thoroughly dry. At the same time 34 unsprayed plants were inoculated, 17 while dry and 17 immediately after sprinkling with tap water. All of the plants were shaded from the sun until the following day. At the end of 19 days after inoculation 95 per cent of the unsprayed plants inoculated when wet had contracted the wilt and 88 per cent of those inoculated when dry. In the sprayed plants there was 33 per cent of infection among those inoculated before drying and 36 per cent among those inoculated after drying.

As will be seen, the percentage relations between infection in wet and dry leaves vary inversely in the sprayed and unsprayed plants. The difference is small, but it occurs in the direction to be expected from known facts concerning conditions favorable to infection. In the absence of bactericidal substances a moist leaf surface presents a better environment for infection by the bacteria; but when a bactericide is present which is effective in solution the maximum effect occurs in the presence of water. This is exactly the result obtained in the experiment under discussion.

SUMMARY

(1) In fields where wilt had largely destroyed the cucumber crop during the preceding season the disease did not appear in 1915 on cucumbers in 48 beetle-proof cages. On the other hand, wilt was very prevalent in those fields on all sides of the cages. In a large number of greenhouse tests where one out of two plants in a pot was inoculated and wilted to the ground the second plant in no case contracted the wilt. The inoculations by means of bacterial suspensions poured on the soil around potted cucumber plants showed a small but varying percentage of wilt. Root injuries were found in most of these cases of root infection. Apparently infection does not enter the uninjured root system from the soil.

(2) In all cases seeds from diseased fruits failed to produce diseased plants, and cultures from such seeds in no case gave the wilt organism, but further tests should be made.

(3) In the tests made stomatal infection did not occur.

(4) The experiments thus far completed show that cucumber beetles (*Diabrotica* spp.) are the most important, if not the only, summer carriers of the wilt organism (*Bacillus tracheiphilus*) and that at least one species (*D. vittata*) is capable of carrying the wilt over winter and infecting the spring planting of cucumbers. In the tests by the writers the squash bug (*Anasa tristis*), the flea beetle (*Crepidodera cucumeris*), the melon aphis (*Aphis gossypii*), and the twelve-spotted lady-beetle (*Epilachna borealis*) have failed to transmit the disease.

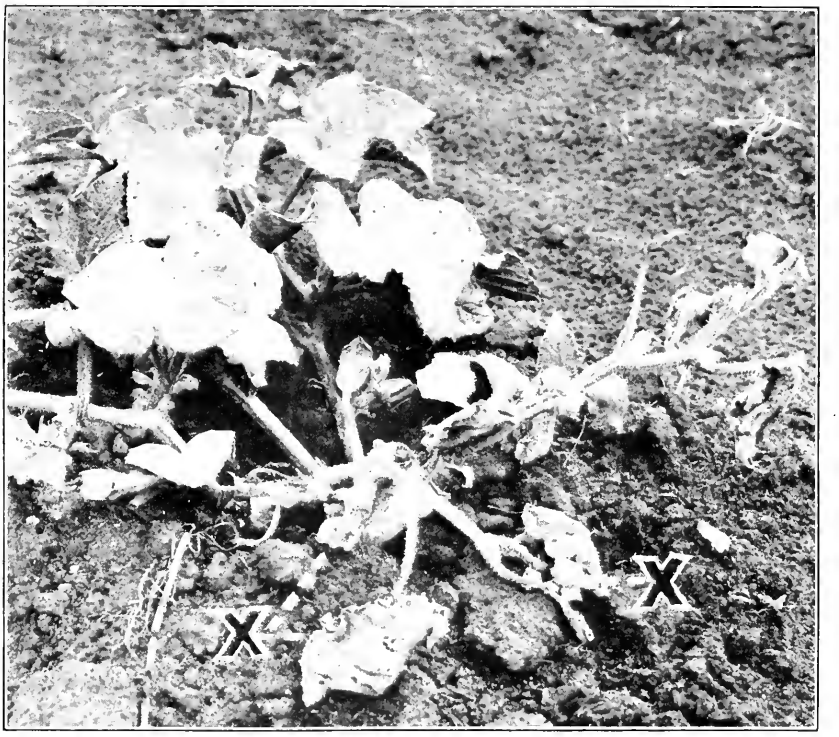
(5) In the field experiments during one season with many different varieties of cucurbits, the greatest difference in resistance was shown by varieties of squash, in which the percentage of infection varied from 0 to 100. The varieties of cucumber and cantaloupe, while showing some difference in their susceptibility to the wilt, give much less promise of control by varietal resistance.

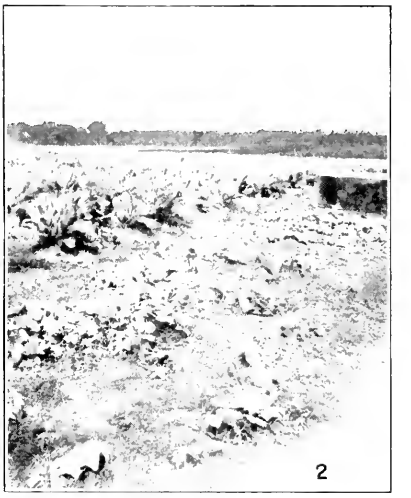
(6) In the spraying experiments of 1915 wilt was effectively controlled by early treatments with a combination of Bordeaux mixture and arsenate of lead. Plots sprayed with either mixture alone showed much less wilt than unsprayed plots, but control was not as complete as where the two were used together. Both field observations and greenhouse experiments indicate that the wilt control is effected through the bactericidal action of Bordeaux mixture, the insecticidal action of arsenate of lead, and the repellent action of both against the cucumber beetles.

(7) Inasmuch as it has been definitely proven that the striped cucumber beetle (*D. vittata*), and also the twelve-spotted cucumber beetle (*D. duodecimpunctata*), are the most active carriers of the bacterial wilt, it becomes necessary to control the insects in order to prevent the disease. This phase of the work will be actively undertaken in cooperation with the Bureau of Entomology during the coming season.

PLATE LIII

Two wilted cucumber plants which contracted bacterial wilt at beetle gnawings of the leaves marked *x*. Three healthy, uninjured plants in same hill are also shown. From field 1, East Marion, Long Island, N. Y., July 19, 1915.


X
X


2


3


4

PLATE LIV

Plots in field 1, East Marion, Long Island, N. Y., 1915. The poor stand in figures 2 and 3 was due entirely to bacterial wilt.

Fig. 1.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning June 25. Photographed September 20, 1915.

Fig. 2.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning July 19, after most of the striped-beetle injury had occurred. Photographed September 20, 1915.

Fig. 3.—Plot sprayed with Bordeaux mixture and lead arsenate, beginning July 27. Practically no beetle injury occurred after this date. Photographed September 20, 1915.

Fig. 4.—General view of field, showing cages and meteorological-instrument shelter. Photographed July 10, 1915.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

15 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

▽

Vol. VI JUNE 19, 1916 No. 12

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., JUNE 19, 1916 NO. 12

CORRELATED CHARACTERS IN MAIZE BREEDING

By G. N. COLLINS,

Botanist, Office of Acclimatization and Adaptation of Crop Plants, Bureau of Plant Industry

INTRODUCTION

The study of correlations as an aid to plant breeding was at one time thought to be full of promise, but in recent years little use has been made of correlations by practical workers. From this fact it might appear that the early hopes were unwarranted, and that correlation is a factor of little or no importance. It must be conceded that the elaborate calculations of correlation coefficients have in few instances proved of value to the practical breeder, yet it must be admitted on reflection that nearly all successful breeding has in reality been made possible by the fact that correlations exist.

In plant breeding the improvement and preservation of varieties has largely resulted from the ability of the breeder to recognize desirable types, and the existence of definite types is in itself a manifestation of the correlation of characters. The existence of types must mean that there are many individuals that present approximately the same combination of characters, and this is exactly what correlation implies. The characteristics of the desired type are recognized by the breeder even though they may not be formulated, and varieties are seldom established by selection confined to a single character. If the study of correlations has appeared to have little bearing on plant breeding, it must be that we have been studying the wrong characters or studying them in the wrong way.

In the improvement of maize varieties (*Zea mays*), as with other plants, the recognition of types has been an important factor. The selection, however, has been almost entirely confined to the ear. In a field of any commercial variety it is easy to recognize differences in the plants, but even after long familiarity with the variety the plants refuse to be classified into distinct groups. This difficulty in recognizing types among maize plants greatly increases the difficulty of breeding this crop.

The lack of recognizable types in maize is very different from the condition that obtains, for example, in cotton (*Gossypium* spp). With cotton,

skilled breeders are able to detect deviation from type even in the early stages of development and the practice of roguing can proceed with certainty. It appears that when a cotton plant deviates from type it deviates in a more or less definite way and in many particulars, or, in other words, there are a number of coherent or correlated characters.

It seemed desirable to determine whether the difficulty in recognizing types in maize is due to a lack of familiarity with the plants or whether there is a fundamental difference between the heredity of maize and that, for example, of cotton.

In the seed characters of maize a definite correlation has been found between the color of the aleurone and the texture of the endosperm (Collins and Kempton, 1913). Correlations have also been noted between the color of the silk and the color of the anthers (Webber, 1906), and between the color of the seed and the color of the cob, dwarfness and broad leaves, and between stamens in the ear and club-shaped tassels (Emerson, 1911). There was, therefore, abundant reason for suspecting that the difficulty of recognizing types among maize plants might be due to a lack of sufficient discrimination, and it was with the idea that correlations were the rule rather than the exception that the present experiment was undertaken. Contrary to expectation, the results give evidence that for the varieties and characters studied there is almost a complete absence of genetic correlations.

CLASSIFICATION OF CORRELATIONS

Correlations may be classified in a great variety of ways and with almost any degree of refinement. As with any classification of organic activities, no particular grouping can be made to serve all purposes, for it is necessary to divide the subject in different planes.

For purposes of the present discussion correlations, or the mutual relations of characters, are divided into three main groups, to which the names "physical," "physiological," and "genetic" may be applied.

PHYSICAL CORRELATIONS are those in which the relation is obviously causal. In many instances correlations of this kind are little more than different names for the same phenomenon, or parts of the same phenomenon, as when increased weight is correlated with increased height. In physical language one of the characters would be described as a function of the other.

PHYSIOLOGICAL CORRELATIONS are those where both characters are the result of the same physiological tendency, as when long internodes in the main stalk are correlated with long internodes in the branches. This may be looked upon as a general tendency to elongated growth that is manifested in different parts of the plant.

GENETIC CORRELATIONS cover the large residue of correlations, the nature and causes of which are questions of controversy, but which are

associated with the method or mechanism of heredity. An example of this type of correlation is shown in the association of yellow petals and deeply lobed leaves in Egyptian × Upland cotton hybrids.

This classification differs from those proposed by Webber (1906) and East (1908) chiefly in placing physical correlations outside the pale of biological correlations. Most of those correlations classed by Webber as morphological would here be considered as physical. This distinction is made because it seems to the writer that the relation between length and weight, for example, is inherent in the properties of matter and is not a biological phenomenon. Certainly a relation of this kind would be found in stones or any inanimate objects selected at random.

Since physiological functions are always directly or indirectly induced by or at least associated with environmental stimuli, Webber's environmental and physiological correlations are here combined. That the examples of physiological correlations cited by Webber are reverse or negative correlations need not confuse the issue, since by simply stating the relation in other terms the correlations can be made to appear as positive.

The distinction between physiological and genetic correlations may not always be easy to apply, and the apparent need of it may disappear entirely with a more complete knowledge of inheritance and methods of growth. For the present, however, the distinction will be useful even if physiological correlations are confined to pure lines or asexually propagated stocks where differences in inheritance can be eliminated. To ascribe the long internodes of the main stem and branches to the activity of a single determiner or gene is hardly less futile than to offer the same explanation for the correlation between the length and weight of inanimate objects. If the one is inherent in the properties of matter, the other is inherent in the properties of plants.

All examples of genetic correlation are exceptions to the third law of Mendel, which implies that characters are redistributed in the perjugate generations of a hybrid in accordance with the laws of chance. Conversely, all instances in which Mendelian ratios, other than the 3 to 1 ratio of a monohybrid, are followed with exactness demonstrate the action of this third law and the absence of correlations among the factors which make up the characters. It should be kept in mind, however, that multiple hybrid ratios have seldom been determined with any great degree of accuracy, so that correlations, unless of a pronounced type, would escape detection.

The significant factor in genetic correlations is the grouping of the characters in the ancestry and not the inherent properties of the characters themselves. Thus, when colored aleurone and horny endosperm are found to be correlated in the progeny of a hybrid, involving colored and white aleurone and horny and waxy endosperm, it does not indicate

any attraction between colored aleurone and horny endosperm, but rather that one of the parents had colored aleurone and horny endosperm, while the other parent had white aleurone and waxy endosperm. This tendency for parental combinations to reappear has been called "coherence," and, so far as known, all genetic correlations thus far recorded are of this nature.

Many investigations have been devoted to correlations in agricultural plants, but unless the special class of correlations covered by coherence is kept in mind the results are likely to be disappointing to the breeder. Cylindrical ears of maize may be correlated with high yield in one population and the opposite result be reached in another case, depending on whether these characters were introduced into the population under investigation from the same parent or from different parents.

There are doubtless many physiological correlations that may be detected by elaborate measurements, but unless the observations are confined to asexually propagated groups or to those of which the ancestry has been carefully studied, there will always remain the uncertainty whether there is an inherent physiological relation between the development of the two characters or whether the correlation is the result of ancestral combinations. The distinction is not without practical importance, for a physiological correlation can not be reversed by any direct means at the disposal of the breeder—that is, without evoking mutation or some form of evolutionary change—while, if the correlation is genetic, the relation between the characters may usually be reversed by a few generations of selection in the desired direction.

Two principal theories have been advanced to explain genetic correlations. These are the theory of reduplication (Bateson, Saunders, and Punnett, 1906) and the theory of linkage developed by Morgan and his students (1915) from studies of the fruit fly *Drosophila ampelophila*. Both of these theories deal with characters which are alternative, both having been derived from the study of Mendelian inheritance.

With the idea that continuous inheritance is to be looked upon as a complicated form of alternative inheritance, it should be interesting to learn what light the study of genetic correlations between characters that are blended in inheritance may throw on the theories of reduplication and linkage. The experiments described below constitute a preliminary attempt to extend the study of genetic correlations to characters that are continuously inherited.

METHODS OF DISTINGUISHING BETWEEN PHYSIOLOGICAL AND GENERIC CORRELATIONS

To determine with certainty that a given correlation is physiological and not genetic, it would be necessary to demonstrate the existence of the correlation in material where all the individuals possessed the same

hereditary tendencies with respect to the characters studied. Theoretically this is only possible in asexually propagated groups. Approximately pure lines may be obtained where self-pollination is possible, so that if correlations are found they may with assurance be considered physiological. In maize, however, even to approximate pure lines produces such abnormal conditions that some other method of study must be sought.

Even in maize it would seem that the question might be approached by comparing the degree of correlation in types or varieties having a relatively restricted ancestry with that observed in the perjugate generations of hybrids between two contrasting forms.

An equally satisfactory method is to compare the degree of correlation in the first or conjugate generation of a hybrid with that of the perjugate generations. Where the conjugate generation is all descended from a single cross, the gametic differences should be no greater than self-pollinated progenies of the parents.

Unfortunately in our experiment the number of first-generation individuals was too limited to detect any but relatively large correlations. Wherever data were available, additional evidence has been presented from the behavior of the original varieties. Although a large number of plants of both parent varieties have been grown and measured, the data have been secured in different localities and in different years, a fact that renders many of the measurements unavailable for these studies.

DESCRIPTION OF MATERIAL

The hybrid that afforded the data for the present paper was a cross between Waxy Chinese and Esperanza, two varieties of maize separated by a number of definitely contrasted characters. The hybrid was made at Lanham, Md., in 1908.

The peculiarities of the Waxy Chinese variety (Pl. LV–LVI) have been described elsewhere (Collins, 1909).

The particular plant used as female parent of the hybrid was grown from the original seed imported from China. The individual notes taken in 1908 give the following details:

> Height, 167 cm. Length of fifth leaf from the top, 83 cm. Width of fifth leaf, 9 cm. Leaf sheath smooth. Nodes above the ear, 4. Suckers, 0. Plant rather open, but distinctly one-sided.

The Esperanza variety belongs to a peculiar type of maize that appears to be confined to the table-lands of Mexico, the *Zea hirta* of Bonafous (1829). This variety was obtained in 1906 from Esperanza, Pueblo, Mexico, by Mr. H. Pittier, of the Bureau of Plant Industry (Pl. LVIII and LIX).

The plant that was the male parent of the hybrid was raised from seed grown at Lanham, Md., in 1907. Regarding the 1907 plants, the notes

state that all were typical of the hairy Mexican type, ranging from 150 to 210 cm. in height. The notes recorded for the 1908 plant used in making the hybrid state that it was typical of the variety except for a general shortening of the internodes. It was 105 cm. high, had three tassel branches, four nodes above the ear, and the fifth leaf from the top measured 83 by 14 cm.

Sixteen first-generation plants were grown in 1909. Three pure-seed ears that provided seed for the second generation are designated as No. 1, 2, and 3. Four plants entered into the parentage of these three ears. No. 1 and 2 were reciprocals. No. 1 resulted from pollinating plant 225 by plant 226. No. 2 by pollinating plant 226 by plant 225. No. 3 was the result of pollinating plant 262 by plant 263. The ears on all three of the first-generation plants that produced ears 1, 2, and 3 showed the usual mixture of waxy and horny seeds that result from crossing the Waxy Chinese and a corneous or horny variety. The notes taken on the four first-generation plants are presented in Table I.

TABLE I.—*Description of four first-generation maize plants grown in 1909*

Plant No.	225	226	262	263
Height.....cm..	222	228	212	230
Number of tassel branches.....	20	.....	18	17
Nodes above the ear.....	5	5	5	5
Length of fifth leaf.....cm..	14	13	14	13.5
Width of fifth leaf.....mm..	63	76	86	88
Exsert of tassel.....	.....	0	0	(a)
Arrangement of leaves.....	(b)	(b)	(b)	(c)

[a] Exserted. [b] Scorpioid. [c] Neither monostichous nor scorpioid.

The final planting was made in 1914. The remnant of seed from the original hybrid ear was planted and furnished 31 first-generation plants. Six rows of approximately 30 plants each were secured of second-generation plants, one row from waxy, and one from the horny seeds of each of the first-generation ears.

The means of the characters measured are given in Table II, and the coefficients of variation in Table III.

TABLE II.—*Mean of different characters in first- and second-generation maize plants*

Character.	First generation.	Second generation.					
		Ear 1.		Ear 2.		Ear 3.	
		Plants from waxy seed.	Plants from horny seed.	Plants from waxy seed.	Plants from horny seed.	Plants from waxy seed.	Plants from horny seed.
Height ..... cm.	223.0 ±2.26	195.0 ±4.12	194.0 ±3.32	192.0 ±3.83	190.0 ±4.70	182.0 ±4.59	185.0 ±4.75
Length of branching space ..... cm.	15.0 ± .37	13.9 ± .37	13.4 ± .33	13.6 ± .29	13.2 ± .32	16.5 ± .58	13.8 ± .42
Length of central spike ..... cm.	29.5 ± .54	26.8 ± .76	27.4 ± .57	25.8 ± .49	25.4 ± .58	24.1 ± .46	24.8 ± .37
Number of branches	28.1 ± .94	37.1 ±1.31	35.1 ±1.14	35.0 ± .84	35.4 ±1.02	31.7 ±1.14	28.9 ±1.27
Number of secondaries	5.3 ± .31	6.9 ± .37	5.9 ± .32	5.3 ± .32	6.3 ± .35	3.6 ± .37	3.6 ± .41
Number of nodes above ear	4.8 ± .88	4.7 ± .11	4.6 ± .10	4.7 ± .06	4.2 ± .08	4.2 ± .06	4.6 ± .07
Length of longest leaf ..... cm.	114.0 ± .64	109.0 ±1.16	107.0 ±1.09	108.0 ± .99	107.0 ±1.33	99.0 ±1.47	104.0 ±1.40
Width of longest leaf ..... cm.	12.7 ± .16	11.9 ± .17	11.9 ± .22	12.0 ± .17	12.5 ± .133	10.9 ± .297	11.4 ± .220
Ratio of length to width	9.05± .108	9.24± .134	9.15± .174	9.04± .127	8.59± .118	9.25± .108	9.16± .182
Number of nodes above longest leaf	8.6 ± .13	8.7 ± .21	8.1 ± .18	8.8 ± .17	8.1 ± .23	7.3 ± .21	8.1 ± .17
Total number of nodes	24.1 ± .20	24.8 ± .40	.....	24.5 ± .24	23.7 ± .32	22.4 ± .31	22.2 ± .20
Number of sheaths with hairs	9.5 ± .17	8.4 ± .29	8.8 ± .22	7.8 ± .32	8.1 ± .28	6.4 ± .22	7.5 ± .22
Number of sheaths encircled by hairs	2.9 ± .36	1.6 ± .37	3.1 ± .32	1.4 ± .32	2.4 ± .41	.2 ± .08	1.0 ± .26
Length of hairs ..... mm.	4.9 ± .14	4.9 ± .13	4.7 ± .10	4.8 ± .12	4.8 ± .64	4.5 ± .13	4.7 ± .16
Deusity of spikelets	34.0 ±1.31	31.2 ± .85	32.6 ±1.46	32.6 ±1.33	31.9 ± .82	35.3 ±1.19	39.5 ±1.52
Length of glumes ..... mm.	9.3 ± .12	9.6 ± .12	10.1 ± .08	9.8 ± .14	10.2 ± .14	9.0 ± .14	8.5 ± .20
Number of erect blades	2.7 ± .23	2.4 ± .24	2.5 ± .25	3.1 ± .34	2.6 ± .32	2.4 ± .22	2.7 ± .24
Angle of tassel axis ..... (°)	44.3 ±3.78	46.0 ±6.30	42.0 ±3.49	37.0 ±3.80	42.5 ±4.93	45.3 ±5.62	36.2 ±5.67
One-sidedness	5.4 ± .38	4.1 ± .28	3.9 ± .33	4.9 ± .37	4.2 ± .30	4.5 ± .31	4.0 ± .3
Number of rows, upper ear	18.3 ± .36	18.5 ± .64	19.6 ± .48	19.0 ± .41	18.3 ± .36	17.1 ± .35	17.9 ± .48
Number of rows, second ear	17.2 ± .52	19.8 ± .73	18.2 ± .86	17.8 ± .56	17.4 ± .51	15.1 ± .50	16.7 ± .46
Number of husks, upper ear	12.9 ± .25	11.1 ± .40	11.2 ± .31	11.0 ± .32	11.3 ± .33	11.8 ± .30	11.6 ± .41
Number of husks, second ear	12.7 ± .35	10.3 ± .54	11.3 ± .37	9.7 ± .25	11.7 ± .38	11.9 ± .42	11.8 ± .41
Exsert of tassel ..... cm.	− 3.05 ± .58	− 2.77± .41	− 3.17± .40	− 3.05± .33	− 2.68± .45	− .353± .87	− .063 ±1.02

TABLE III.—*Coefficient of variation of characters in first- and second-generation maize plants*

Character.	First generation.	Second generation.					
		Ear 1.		Ear 2.		Ear 3.	
		Plants from waxy seed.	Plants from horny seed.	Plants from waxy seed.	Plants from horny seed.	Plants from waxy seed.	Plants from horny seed.
Height.....cm..	8.0± 0.7	14.7± 1.5	13.0± 1.1	14.5± 1.4	19.0± 1.8	14.9± 1.8	17.0± 1.8
Length of branching space.....cm..	19.3± 1.8	20.4± 1.9	19.6± 1.8	15.8± 1.5	18.3± 1.7	22.0± 2.5	20.6± 2.2
Length of central spike, cm.....	14.5± 1.3	19.7± 2.0	15.2± 1.5	13.8± 1.4	17.5± 1.6	11.6± 1.4	9.7± 1.0
Number of branches.....	26.3± 2.5	27.0± 2.7	25.2± 2.4	17.5± 1.7	22.0± 2.1	22.0± 2.7	29.1± 3.3
Number of secondaries..	46.4± 4.9	40.4± 4.2	42.4± 4.5	43.6± 4.7	41.9± 4.6	62.0± 9.1	76.2±11.6
Number of nodes above ear.....	14.4± 1.3	17.4± 1.7	17.3± 1.5	9.6± 1.0	14.3± 1.4	8.3± .9	10.4± 1.1
Length of longest leaf, cm.....	4.4± .4	8.1± .7	8.2± .7	6.7± .8	9.7± 1.0	8.7± .9	9.4± 1.0
Width of longest leaf, cm.....	10.0± 2.5	10.7± 1.0	15.1± 1.3	10.4± 1.0	8.2± .7	12.8± 1.3	17.4± 1.9
Ratio of length to width.	9.3± .8	11.0± 1.0	10.7± 1.0	10.2± 1.0	10.5± .9	12.9± 1.4	11.7± 1.3
Number of nodes above longest leaf.....	12.1± 1.1	18.4± 1.7	17.5± 1.6	14.2± 1.4	21.8± 1.9	18.8± 2.0	13.1± 1.4
Total number of nodes..	6.2± .6	10.9± 1.1	.....	6.8± .7	9.1± .9	8.0± 1.0	5.7± .7
Number of sheaths with hairs.....	14.1± 1.2	26.2± 2.5	19.8± 1.8	29.4± 3.2	26.4± 2.5	23.3± 2.5	19.1± 2.1
Number of sheaths encircled by hairs.....	100.0±15.0	170.0±42.0	83.4±11.1	169.0±42.0	132.0±25.0	225.0±78.2	175.0±50.0
Length of hairs....mm..	22.4± 2.0	19.7± 1.9	17.2± 1.5	18.5± 1.9	10.4± 1.0	18.6± 2.3	21.3± 2.3
Density of spikelets.....	30.3± 2.9	20.2± 1.9	36.2± 3.5	29.7± 3.1	19.8± 1.9	21.6± 2.4	24.8± 2.8
Length of glumes..mm..	10.7± 1.0	9.1± .9	6.8± .6	10.2± 1.0	10.5± 1.0	9.1± 1.0	15.5± 1.7
Number of erect blades..	57.4± 6.9	68.7± 9.8	66.9± 9.5	60.6±10.1	75.0± 9.8	54.2± 8.0	51.3± 7.5
Angle of tassel axis...(°)..	67.2± 8.9	98.2±16.5	66.7± 7.9	74.4±10.4	89.5±13.1	77.9±12.8	101.0±18.6
One-sidedness.....	53.6± 6.4	48.8± 5.7	56.5± 7.5	49.6± 6.6	52.6± 6.4	46.4± 5.9	47.8± 6.3
Number of rows, upper ear.....	13.7± 1.4	20.7± 2.5	17.2± 1.7	13.8± 1.8	13.7± 1.4	12.7± 1.4	16.3± 1.9
Number of rows, lower ear.....	18.6± 2.0	15.5± 2.5	19.9± 3.3	12.4± 1.6	15.0± 2.0	13.8± 1.7	13.0± 1.9
Number of husks, upper ear.....	15.1± 1.4	24.6± 2.6	20.6± 2.0	18.8± 2.0	20.2± 2.1	15.3± 1.8	22.7± 2.6
Number of husks, lower ear.....	19.3± 2.0	23.1± 3.6	14.4± 2.2	11.6± 1.8	19.5± 2.4	15.8± 2.5	18.0± 2.5

A comparison of these tables shows that the first-generation plants exceeded the second-generation plants in height, length of branching space, length of central spike, length and width of longest leaf, number of nodes above the longest leaf, number of leaf sheaths with hairs, and number of single-ranked blades. The second-generation plants exceeded the first-generation plants in the number of tassel branches. In the other characters there was no significant difference between the means of the first and second generation plants.

The first-generation plants were distinctly less variable than the second-generation plants in height, length, and width of longest leaf, number of nodes above the longest leaf, total number of nodes, and number of leaf sheaths with hairs. The first-generation plants were more variable with respect to the length of the tuberculate hairs and density of spikelets.

The least variable character measured was the length of the longest leaf. The total number of nodes was also comparatively uniform. The very high coefficient of variation for the number of sheaths encircled by hairs results in part from the alternative nature of this character.

In the progeny of the reciprocal ears 1 and 2 there are no really significant differences. The progeny of ear 3, however, which descended from entirely different first-generation plants, shows a number of differences from the remainder of the second-generation plants.

Although the average height of the plants from all these ears is practically the same, the progeny of ear 3 shows smaller values for a number of other dimensional characters. The number of branches, primary and secondary, length of leaf, total nodes, length of glumes, and number of rows of grains are all slightly lower. With the exception of length of leaf and length of glumes, these differences might be interpreted as indicating a more pronounced development of the Esperanza characters. The same may be said of the exsert, which is higher in ear 3. In the development of tuberculate hairs, on the other hand, the progeny of ear 3 was decidedly more like the Chinese variety.

In addition to the measurements given in Tables II and III, there are a number of differences that deserve to be more fully discussed.

HAIRS ON THE LEAF SHEATH

Perhaps the most striking difference between the varieties is the covering of the leaf sheaths. In the Chinese variety the leaf sheaths are similar to those of the ordinary types of maize. The surface is smooth, except for fine spicules, which occur especially over the fibrovascular bundles. The spaces between the fibrovascular bundles are crossed by numerous diagonal ridges or cross veins irregularly arranged and usually discontinuous at the fibrovascular bundles. These cross veins with the fibrovascular bundles cover the surface of the sheath with a coarse reticulum.

In the Esperanza variety the cross veins of the sheaths are absent or confined to the seedling leaves, and the spaces between the bundles are occupied by tubercles, each bearing a long hair (Pl. LVIII). These tuberculate hairs are absent from the sheath of the first six to eight leaves of the seedling. They appear abruptly and may cover the entire surface of the first sheath on which they appear. The hairs are from 3 to 5 mm. long, and the tubercle is approximately ½ mm. wide and of the same height.

In the Waxy Chinese variety tuberculate hairs are completely absent (Pl. LVI, fig. 2). As in all varieties, there is a small area closely confined to the throat of the sheath that is clothed with long hairs. It is not clear whether these hairs are homologous to the tuberculate hairs of the Esperanza variety or not. Even considering these hairs at the throat of the leaf sheath in the Waxy Chinese variety as of the same type, the two varieties are completely separated, with not even an approach to overlapping forms. In the hybrid and its progeny three methods of measuring the degree of hairiness were employed:

(1) By recording the total number of nodes with hairy sheaths.

(2) By recording the number of nodes with hairs completely encircling the sheath. In the pure Esperanza maize this usually occurred at the lowest node on which hairs were borne; or at most there was a difference of only one of two nodes. In the hybrid plants there were usually a number of sheaths with tuberculate hairs at the side, but with a narrow smooth strip at the back over the midrib.

(3) By recording the length of the longest tuberculate hairs. In all hybrid plants of both the first and second generation tuberculate hairs were present, there being no plant that resembled pure Waxy Chinese plants in this particular. The length of the hairs varied, however, in different plants, thus affording another measure of the extent to which hairs were developed.

TASSEL CHARACTERS

In the nature of the tassel the two varieties are hardly less distinct than in the covering of the leaf sheath. The Waxy Chinese variety has many branches, 15 to 30 primary branches in normally developed plants, with numerous secondaries. The Esperanza (Pl. LVII) seldom has more than 5 branches and in many plants the tassel is simple, consisting only of a large central spike. Associated with the difference in the number of branches is a corresponding difference in length of the axis or "branching space," the distance from the lowest to the uppermost branch.

In the Esperanza variety the glumes vary from 10 to 16 mm. in length with a mean of 11.7 ± 0.14. In the Waxy Chinese variety the range is from 7 to 12 mm., with a mean of 9.2 ± 0.09. All of the above characters were directly measured or counted.

The typical arrangement of the spikelets is also different in the two varieties. In the Waxy Chinese the arrangement on the branches is similar to that in most of the better known varieties of maize. The spikelets are paired, one pediceled and one sessile, the pairs alternating on the sides of the branch. In the Esperanza maize when branches occur the spikelets are nearly all sessile and are borne in clusters of from 2 to 5. They are also disposed on all faces of the branch instead of being confined to the sides. The arrangement of spikelets and general appearance of the branches in the Esperanza is similar to the arrangement on the central spike. One result of these differences in arrangement of spikelets is a greater crowding of spikelets in the Esperanza. As a measure of this difference the number of spikelets in the last 10 cm. of the lowest tassel branch were counted. This number is referred to as the "density of the spikelets."

TASSEL EXSERT

In the Waxy Chinese variety the base of the tassel is frequently inclosed in the uppermost leaf sheath. In the Esperanza variety the lowest branch of the tassel is usually well above the uppermost leaf

sheath in the mature plant. Differences in this particular were recorded by measuring the distance from the top of the uppermost sheath to the origin of the lowest tassel branch, the measurement being expressed as a minus quantity when the base of the branch was included in the sheath.

This character is especially subject to environmental changes. Unfavorable conditions, such as drought occurring late in the season, will prevent the elongation of the upper internodes to such an extent that all varieties may show a minus exsert. Comparisons must therefore be confined to plants grown in a single season in the same locality.

The range as recorded for Waxy Chinese grown at different times is from -14 cm. to 7 cm., with the mean at -1.31 ± 0.3. In Esperanza the range is from -3 cm. to 18 cm., with the mean at 6.07 ± 0.5.

NUMBER OF ERECT LEAF BLADES

In the Waxy Chinese variety the upper leaf blades are held erect instead of diverging. In ordinary varieties which the Esperanza resembles with respect to this character the upper leaf blades make approximately a right angle with the axis (Pl. LV, LVII). As a measure of this character the number of erect leaf blades was recorded. For example, if the two uppermost leaves were erect and the third leaf was the first to exhibit an angle, the plant was classed as 2, with respect to this character.

Recorded in this way there would be some overlapping in the parent varieties, since in some Waxy Chinese plants even the uppermost leaf shows an appreciable angle. In reality, however, the two types are distinct, for in the Esperanza not only is the uppermost leaf never erect, but it is seldom borne at less than a right angle with the stalk.

ANGLE OF TASSEL AXIS

In the Esperanza variety the tassel is always erect. In the Waxy Chinese plant the tassel is usually curved or declined (Pl. LV, LVII). This character is variable in the Chinese, some plants having the tassel perfectly erect. The tendency, however, to an inclined tassel, as it appears in the hybrid, may properly be ascribed entirely to the Chinese variety, no similar tendency ever having been observed in any Esperanza plant. The character was measured by estimating the angle which the branching space, or that portion of the axis of the tassel between the lowest and highest branch, made with the main stalk. In the pure Waxy Chinese variety this character appears definitely associated or physiologically correlated with the following character of "one-sidedness."

ONE-SIDEDNESS

One of the most striking peculiarities of the Waxy Chinese variety of maize is the displacement of the leaf blades from the usual distichous arrangement, with the result that a number of the upper leaf blades are

borne on one side of the plant instead of alternately on opposite sides of the culm (Pl. LVI, fig. 1). Like the angle of the tassel, this character is not universally present in the Waxy Chinese plant, but, on the other hand, no tendency of this kind has ever been observed in the Esperanza variety.

When one-sided plants occur in the hybrid generations, it is therefore reasonable to assume that the character was derived from the Chinese parent. Measurements of these characters in the hybrid plants were made by recording the number of monostichous or single-ranked leaves.

A recapitulation of the more definitely contrasting characters of the two parent varieties is here presented in parallel columns:

Esperanza variety	Waxy Chinese variety
Horny endosperm.	Waxy endosperm.
Branching space short.	Branching space long.
Tassel erect.	Tassel curved.
Spikelets in clusters.	Spikelets in pairs.
Glumes long.	Glumes short.
Leaf sheaths with tuberculate hairs.	Leaf sheaths without tuberculate hairs.
Upper leaf blades horizontal.	Upper leaf blades erect.
Upper leaf blades distichous.	Upper leaf blades monostichous.

If the characters of maize were subject to coherence, the second generation of a cross between two such diverse and long-established types as Esperanza and Waxy Chinese would seem a most favorable opportunity for its manifestation.

In the whole series of second-generation plants there were none that even approximately represented either parent variety; nor did the plants fall into recognizable groups. With respect to the individual characters, the parental forms reappeared or were even intensified in some instances, but an almost complete and chance reassortment of the characters seems the rule. If the characters were completely independent, a reappearance of the parental types could not, of course, be expected, for, treating the characters as alternative and allowing for only 10 characters, a plant possessing all the characters of either parent could not be expected oftener than once in 10 billion plants. Although the characters themselves, with few exceptions, were non-Mendelian in the sense that they were not alternative, the results conformed to the Mendelian law of recombination. Examples of the combination of characters from the two parent varieties are shown in Plates LIX to LXIII.

Endosperm texture was the only strictly alternative character noted. The number of erect leaves and angle of tassel, while not alternative in the sense of falling into definite groups without intermediates, do, however, approach a Mendelian form of inheritance. The distribution, instead of approximating a normal frequency curve, was distinctly bimodal with respect to these characters. A similar tendency is apparent in the first-generation plants. In connection with this evidence

of segregation in the first generation, it should be recalled that neither of these characters, which belong to the Waxy Chinese variety, is universally present in the plants of that variety, and the parent plant may have been heterozygous. There is also a less pronounced indication in the second-generation plants that one-sideness is Mendelian in its inheritance.

CORRELATIONS

Eleven of the characters most definitely contrasted in the parents were selected and the correlation coefficients between all possible combinations were calculated for both the first and second-generation plants. The results are shown in Table IV. The correlations are so stated that a positive, or plus, correlation indicates a correlation between the characters derived from the same parent; in other words, a coherence. For example, the Waxy Chinese variety has a large number of tassel branches and no tuberculate hairs, while the Esperanza variety has a small number of tassel branches and well-developed tuberculate hairs. In expressing the relation between these two characters, when a large number of tassel branches is found associated with short tuberculate hairs, the correlation is recorded as positive.

Since ears 1 and 2 were reciprocals and no significant differences were found between their progenies, the observed values were used directly in calculating the coefficients of correlation. Where the mean progeny of ear 3 differed from the mean of the combined progenies of ears 1 and 2 with respect to any character, all measurements in the progeny of ear 1 were multiplied by the percentage difference between the means before combining the populations in a correlation table.

The combined progenies of the three first-generation ears numbered 183 individuals. Complete notes could not be taken on all the plants, so that the number of individuals entering into the different correlation tables was reduced to from 125 to 150. Assuming all correlations that are more than 3.5 times the probable error to be worthy of consideration, an examination of Table III shows that 20 of the 55 character pairs fall into this class.[1] With three exceptions the coefficient for the character pairs of this group is 0.2 or larger. Of these 20 character pairs that may be held to show definite correlations in the second generation, 17 are positive—that is, in the nature of coherences—and 3 are negative. All but 5 of the 20 are, however, open to the suspicion of being physiological correlations, since they do not differ materially from the correlations shown for the same characters in the first generation.

The 5 character pairs that show most evidence of genetic correlation are given in Table V. Even here there are no very striking differences between the coefficients of the first and second generations, and it is by no means impossible that even here the differences may be due to chance.

[1] These coefficients are printed in bold-face type in Table IV.

TABLE IV.—*Correlation coefficients*

Characters.	First generation.	Second generation.		
	Coef.	P.E.[1]	Coef.	P.E.[1]	Coef. / P.E.
Small exsert of tassel and: long branching space	0.27	0.13	**0.345**	**0.053**	**6.5**
large number of tassel branches	.30	.13	**.287**	**.055**	**5.2**
large number of erect blades	.22	.14	**.346**	**.059**	**5.9**
high degree of one-sidedness	.00	.15	**.353**	**.056**	**6.3**
large angle of tassel axis	.18	.14	**.411**	**.050**	**8.2**
small number of sheaths with hairs	−.21	.13	−.109	.059	2.0
short hairs	−.39	.12	−.150	.060	2.5
low density of spikelets	−.10	.14	.018	.059	.3
short glumes	−.24	.13	−.036	.059	.6
waxy endosperm	...	...	−.069	.070	.9
Long branching space and: large number of tassel branches	.50	.09	**.442**	**.045**	**9.8**
large number of erect leaf blades	−.22	.14	.202	.061	3.3
high degree of one-sidedness	−.14	.13	**.234**	**.057**	**4.1**
large angle of tassel axis	.00	.13	.170	.055	3.1
small number of sheaths with hairs	.00	.12	.091	.054	1.7
short hairs	−.12	.12	.056	.056	1.0
low density of spikelets	.32	.11	**.198**	**.054**	**3.7**
short glumes	−.01	.12	−.188	.053	3.6
waxy endosperm	...	...	**.242**	**.066**	**3.7**
Large number of tassel branches and: large number of erect blades	−.11	.14	**.222**	**.063**	**3.5**
high degree of one-sidedness	.27	.12	**.221**	**.058**	**3.8**
large angle of tassel axis	.38	.11	**.243**	**.054**	**4.5**
small number of sheaths with hairs	−.30	.11	−.091	.056	1.6
short hairs	.25	.12	−.021	.057	.4
low density of spikelets	.45	.10	**.214**	**.054**	**4.0**
short glumes	.07	.12	−.165	.054	3.0
waxy endosperm	...	...	.104	.067	1.6
Large number of erect leaf blades and: high degree of one-sidedness	.17	.14	**.487**	**.050**	**9.7**
large angle tassel axis	.29	.13	**.513**	**.047**	**10.9**
small number of sheaths with hairs	−.21	.14	−.118	.064	1.8
short hairs	.17	.14	−.081	.063	1.3
low density of spikelets	−.18	.14	.084	.065	1.3
short glumes	.31	.13	.043	.065	1.1
waxy endosperm	...	...	...	.081	...
High degree of one-sidedness and: large angle of tassel axis	.67	.07	**.582**	**.040**	**14.5**
small number of sheaths with hairs	−.15	.13	−.069	.059	1.2
short hairs	.15	.13	.068	.060	1.1
low density of spikelets	.36	.12	.030	.059	.5
short glumes	−.08	.13	−.017	.060	.3
waxy endosperm	...	...	.118	.074	1.6
Large angle tassel axis and: small number of sheaths with hairs	−.23	.11	**−.203**	**.053**	**3.8**
short hairs	.01	.12	.007	.056	.1
low density of spikelets	.02	.13	.123	.055	2.2
short glumes	.47	.10	.043	.056	.8
waxy endosperm	...	...	.037	.079	.5
Small number of sheaths with hairs and: long hairs	−.05	.12	**.193**	**.053**	**3.6**
low density of spikelets	−.01	.12	−.134	.058	2.3
short glumes	.21	.11	−.009	.055	.2
waxy endosperm	...	...	.192	.067	2.9
Short hairs and: low density of spikelets	.12	.12	−.027	.055	.5
short glumes	−.05	.12	.013	.055	.2
waxy endosperm	...	...	−.014	.069	.2
Low density of spikelets and: short glumes	−.16	.12	**−.233**	**.052**	**4.5**
waxy endosperm	...	...	.082	.068	1.2
Short glumes and waxy endosperm	...	...	.177	.059	3.0

[1] P. E. = probable error.

TABLE V.—*Character pairs exhibiting genetic correlations*

Character pair.	Coefficient of correlation.		Difference between first and second generations.	Difference ÷ probable error.
	First generation.	Second generation.		
Small exsert of tassel and one-sidedness	0.00±0.15	0.353±0.056	0.353±0.160	2.2
Branching space and number of erect blades	−.22± .14	.202± .061	.422± .153	2.8
Branching space and one-sidedness	−.14± .13	.234± .057	.374± .142	2.6
Number of tassel branches and number erect	−.11± .14	.222± .063	.332± .143	2.3
Number of erect blades and one-sidedness	.17± .14	.487± .050	.317± .140	2.1

Owing to the small number of first-generation individuals and the consequent uncertainty that attaches to correlation coefficients in that generation, it is, on the other hand, possible that other correlations shown in the second-generation plants are really genetic. From this point of view, it should be noted, however, that 18 of the second-generation correlations are negative.

The possibility of a reduction of physiological correlations must also be considered. The existence of a significant positive correlation in the first generation is taken to indicate a physiological correlation between the characters. With such characters as branching space and the number of branches, the relation is obvious; indeed this relation might almost be classed as physical, since as the branching space approaches zero the number of branches must necessarily become less. There would also appear to be a necessary relation between one-sidedness and angle of the tassel axis, for a perfectly erect tassel could scarcely occur with a high degree of one-sidedness. Where correlations of this nature are lowered in the second generation, it would seem necessary to assume that this reduction is brought about by a tendency for the characters from *different* parents to reappear in the same individual, thus reducing the normal physiological correlation that exists between the characters.

The following are two such character pairs:

	First generation	Second generation	Difference	$\frac{D.}{P.E.}$
One-sidedness and low density of spikelets	0.36±0.12	0.030±0.059	0.330±0.13	2.5
Large angle of tassel axis and short glumes	.47± .10	.043± .056	.427± .114	3.7

It has been mentioned that with respect to both the number of erect leaf blades and the angle of tassel axis there was a tendency for the plants to fall into two groups. This raised a doubt as to the applicability of the customary "product-moment" method of calculating the correlation coefficient where these characters were involved. This group of correlations was therefore recalculated, using Pearson's biserial correlation coefficient (Pearson, 1909). Slightly different values were obtained, but no additional significant correlations were brought to light.

In the second generation the waxy and horny seed were planted separately, thus affording an opportunity for observing whether the plants from seeds having the waxy endosperm characteristic of the Waxy Chinese variety showed any preponderance of other Chinese characters. No consistent differences were apparent in the general appearance of the rows from the waxy and horny seeds. There was such great individual diversity, however, that comparison was difficult. Analysis of the measurements showed little more. The only character that showed a measurable correlation with endosperm texture was the degree to which tuberculate hairs were developed on the leaf sheaths.

Since endosperm texture is strictly alternative, while all other characters were expressed in varying degrees, the method for calculating the correlation coefficients was necessarily different for this group of character pairs. In calculating the correlations with endosperm texture Pearson's (1909) method for calculating a biserial correlation, together with Soper's (1914) formula for the probable error, were used. With a strictly alternative character such as endosperm texture, it would seem impossible to distinguish physiological from genetic correlations. Since one variety always has waxy and the other always has horny endosperm, to detect correlations with this character in the parent varieties seems out of the question. Likewise, as a result of the dominance of the horny endosperm, the seeds from which the first-generation plants were grown were all horny, and there was no opportunity to determine correlations with endosperm texture among first-generation plants.

At the time of planting it was, of course, impossible to distinguish between the seed that were pure for the horny character and those that were heterozygous. An examination of the open-pollinated ears produced by the second-generation plants grown from horny seeds made such a separation possible. All ears that produced any waxy seeds must have grown from heterozygous seeds. No correlations sufficiently large to be detected in the small number of individuals available were found between these two classes and other contrasting characters.

It may be urged that the absence of coherence in the progeny of such a diverse hybrid as the one here discussed may not prove that there is a similar lack of coherence among crossbred individuals within the variety. All maize varieties are, however, of such mixed ancestry that they are in effect hybrid progenies, and even if an exhaustive study of the inheritance of the characters of a narrow-bred variety should show the existence of coherence the results would be beside the point from a practical standpoint, for to maintain a satisfactory degree of vigor in maize a condition of mixed ancestry must be retained.

INTENSIFICATION OF CHARACTERS

The present hybrid affords an interesting sample of an intensified character. One of the peculiarities of the Waxy Chinese variety is the scorpioid top. In plants which exhibit this character the leaf blades of the upper nodes are monostichous and erect, and the tassel is curved to one side. The curving of the tassel was originally interpreted as a direct result of the monostichous arrangement and erect blades. The manner in which this complex of characters reappears in the hybrid with the Esperanza variety shows that, although always associated in pure Chinese maize, they are separable and each may be inherited independently of the others. The curved tassel supposed to be merely the result of the other characters may not only occur alone—that is, in plants with

distichous leaf blades all of which make an angle with the main axis—but the extent of the curving is much greater in some of the hybrid plants than has ever been observed in pure Waxy Chinese plants. The angle of the tassel axis had not been recorded for Waxy Chinese plants before the season of 1915, but thousands of individuals have been observed, and it can be definitely stated that no plant showed a tassel inclined as much as 90° from the perpendicular.

In 148 hybrid plants of the second generation of the hybrid there were 12 plants with the axis of the tassel inclined from the perpendicular by more than 100° and 5 plants having the angle of the tassel axis recorded as more than 145°. The phenomenon is not due to any weakness of the culm, as examples of more than 180° show (Pl. LXII); in fact, the upper part of the culm is particularly thick and rigid, a characteristic of the Chinese parent.

The positiveness of the character was well shown in some of the plants where the curving of the culm caused it to break through the upper leaf sheaths. In such plants the pendent tassels very strongly suggested the "goose neck" of certain sorghum varieties. A plant of this type is shown in Plate LXIII.

CONCLUSIONS

Two principal methods of breeding may be distinguished, depending on the manner in which selection is applied:

(1) Selection may be directed toward the isolation and propagation of desirable types of individuals. The new type may occur as an aberrant individual or as a recognizably distinct strain within the variety, but in either case it is differentiated from the stock by many characters.

(2) Selection is directed to variations of the individual characters regarding which improvement is desired.

With most crop plants the method of selecting types has been by far the most productive, but in the improvement of maize, this method has figured very little. Selection has been by characters instead of by types.

Why the isolation of types of plants has not been a factor in the improvement of maize has not been clear. Though diversities in plant characters are obvious and striking, few breeders have been able to distinguish well-defined types of plants within commercial varieties.

If recognizable types exist it must mean that groups of characters tend to appear together; in other words, the characters are correlated. The extent to which obvious characters are correlated is therefore proposed as a measure of this tendency toward the persistence of types. In the progeny of a hybrid between two very different maize varieties the results here reported show that the characters studied, instead of forming coherent groups, are almost completely independent in inheritance.

By attempting to measure the extent to which types persist by means of correlation coefficients, it is necessary to distinguish different kinds of correlations. For this purpose correlations are here classified as physical, physiological, and genetic. A method is also proposed by which physiological and genetic correlations may be distinguished.

The case studied was a hybrid between two extreme types that must have been completely isolated from very remote times. The large number of well defined characters which differentiate the varieties rendered this material exceptionally favorable for the study of coherence, by which is meant the tendency for characters associated in one of the parents of a hybrid to remain together in the later generation of the hybrid.

For the study of correlations 11 characters were selected in which the parent varieties showed little or no overlapping. The correlation coefficients of all the combinations were calculated, and of the 55 possible combinations 20 were found to exhibit significant correlations. In all but 5 of these, however, the correlations are believed to be physiological rather than genetic. In no instance was there a correlation between two characters closer than 0.5, a fact which in itself offers an explanation of the difficulty of recognizing types in maize.

This lack of coherence of characters in maize, taken with the fact that to maintain a satisfactory degree of vigor a diversified ancestry must be maintained, would appear to make the method of isolating types inapplicable to this plant. As an offset to the limitation thus imposed, advantage may be taken of the facility with which desirable characters derived from different parents can be combined.

LITERATURE CITED

BATESON, William, SAUNDERS, Edith R., and PUNNETT, R. C.
1906. Experimental studies in the physiology of heredity. *In* Rpts. to Evolution Com. Roy. Soc. [London], no. 3, 53 p., 4 fig.

BONAFOUS, Mathieu.
1829. Note sur une nouvelle espèce de maïs. *In* Ann. Sci. Nat., t. 17, p. 156–158, pl. 8.

COLLINS, G. N.
1909. A new type of Indian corn from China. U. S. Dept. Agr. Bur. Plant Indus. Bul. 161, 30 p., 2 pl.

——— and KEMPTON, J. H.
1913. Inheritance of waxy endosperm in hybrids of Chinese maize. *In* IV Conf. Internat. Génétique Paris, Compt. Rend. et Raps., 1911, p. 347–357.

EAST, E. M.
1908. Organic correlations. *In* Amer. Breeders' Assoc. Rpt., v. 4, p. 332–343.

EMERSON, R. A.
1911. Genetic correlation and spurious allelomorphism in maize. *In* Nebr. Agr. Exp. Sta. 24th Ann Rpt. 1910, p. 59–90, 9 fig.

MORGAN, T. H., STURTEVANT, A. H., MULLER, H. J., and BRIDGES, C. B.
1915. The Mechanism of Mendelian Heredity. 262 p., illus. New York. Bibliography, p. 237–256.

PEARSON, Karl.

1909. On a new method of determining correlation between a measured character *A*, and a character *B*, of which only the percentage of cases wherein *B* exceeds (or falls short of) a given intensity is recorded for each grade of *A*. *In* Biometrika, v. 7, pt. 1/2, p. 96–105.

SOPER, H. E.

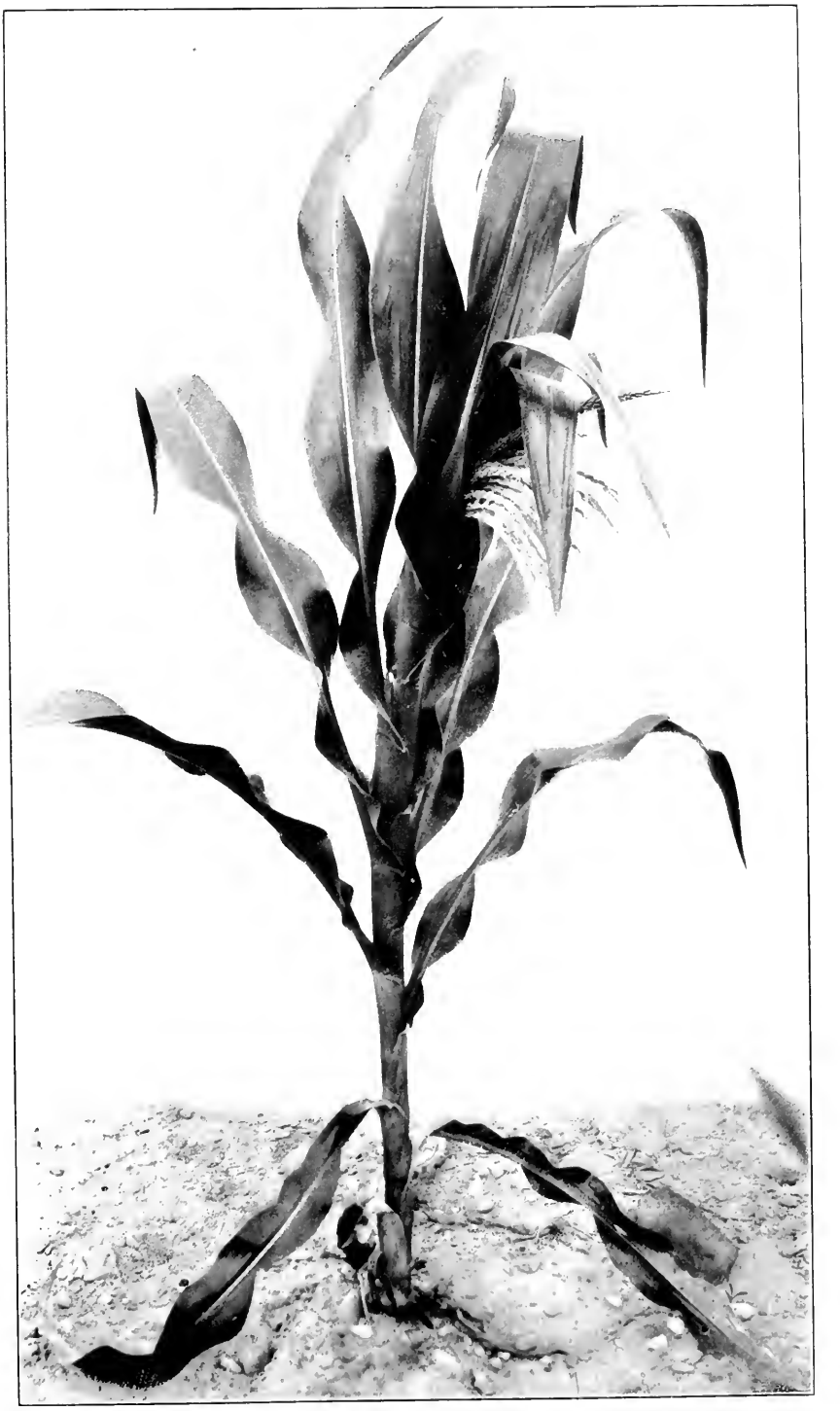
1914. On the probable error of the bi-serial expression for the correlation coefficient. *In* Biometrika, v. 10, pt. 2/3, p. 384–390.

WEBBER, H. J.

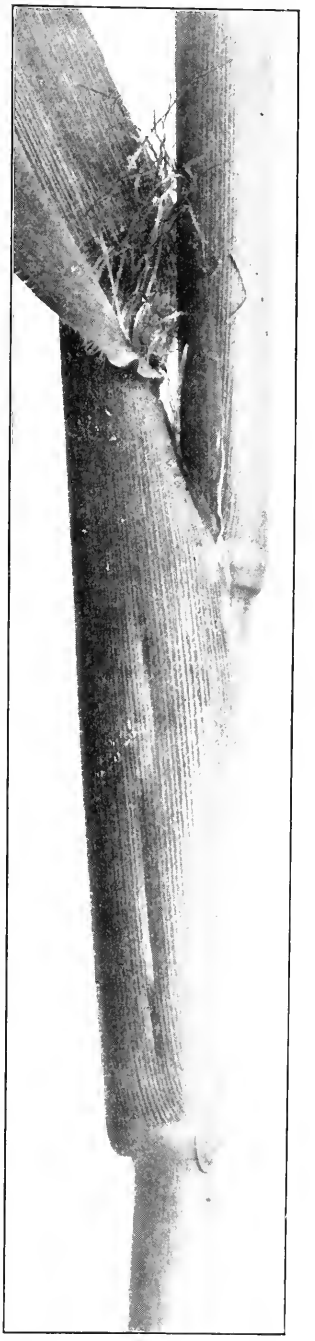
1906. Correlation of characters in plant breeding. *In* Amer. Breeders' Assoc. Rpt., v. 2, p. 73–83, pl. 1.

PLATE LV

Typical plant of the Waxy Chinese variety of maize, showing numerous tassel branches, erect leaf blades, one-sidedness, and curved tassel.



1



2

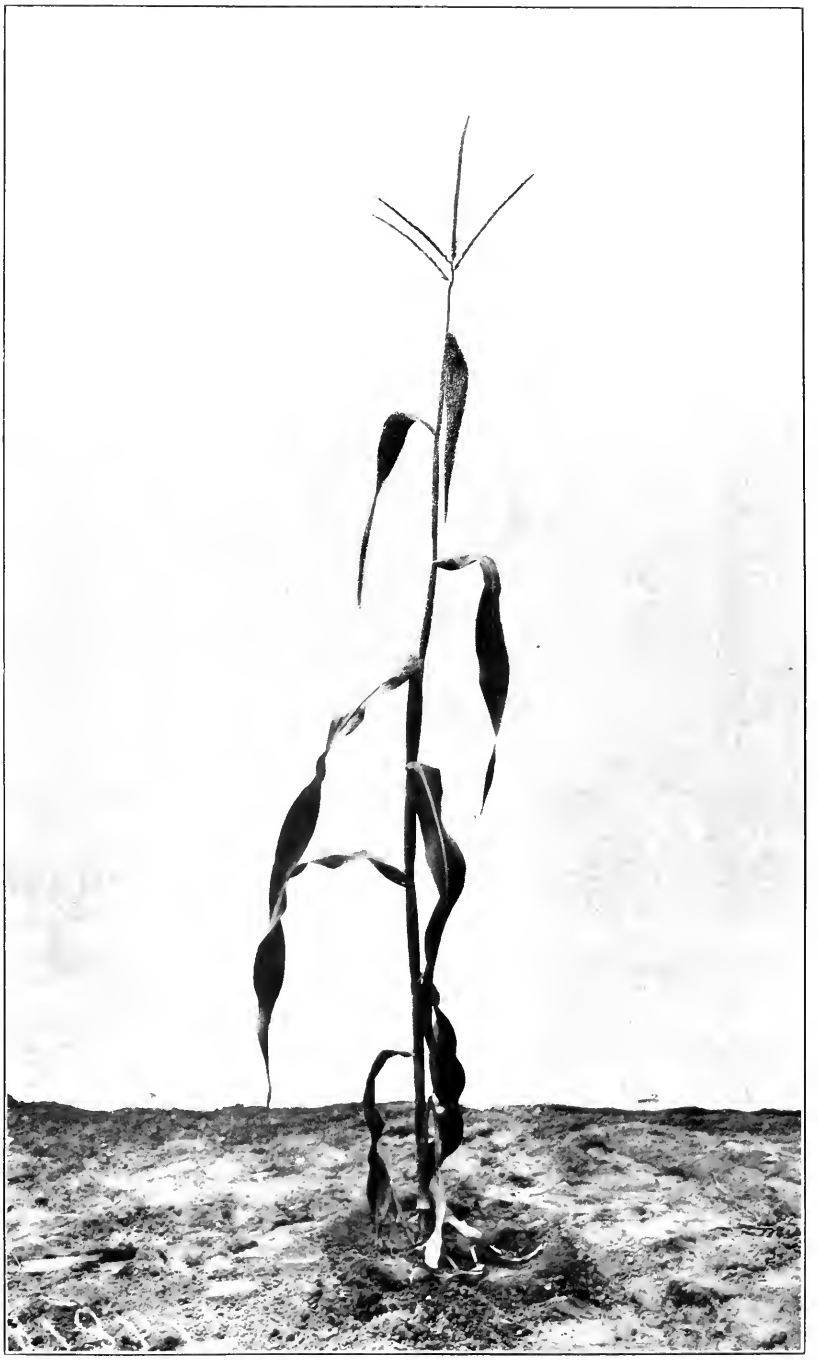
PLATE LVI

Fig. 1.—Uppermost leaf sheaths of Chinese maize plant, showing the one-sided arrangement of leaf blades and absence of hairs. Natural size.

Fig. 2.—Leaf sheath of the Waxy Chinese variety of maize, showing the transverse lines and absence of hairs. Compare with Plate LX. Natural size.

PLATE LVII

A plant of the Esperanza variety of maize, showing the drooping leaves, few tassel branches, and elongated internodes characteristic of the variety.



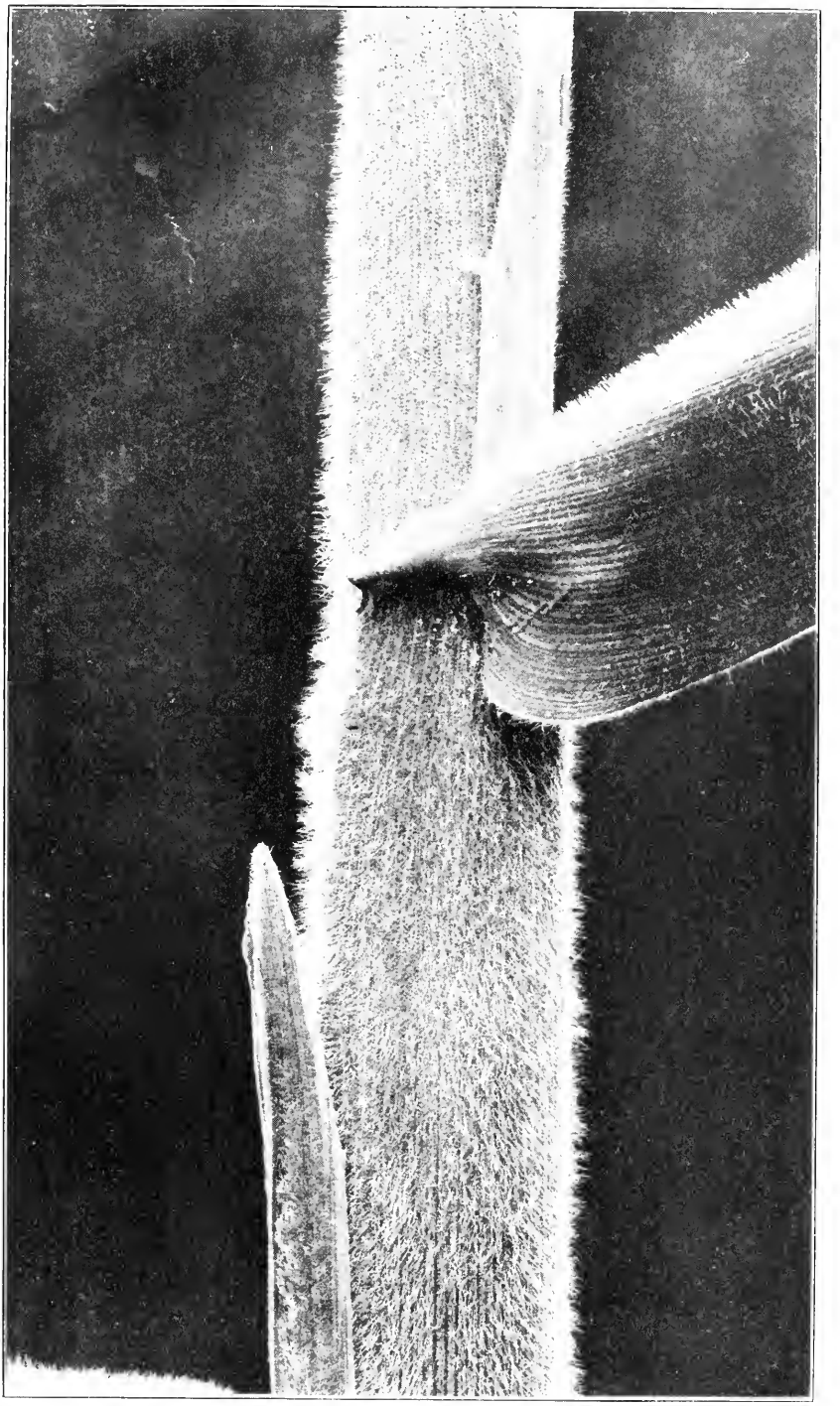


PLATE LVIII

Leaf sheaths of the Esperanza variety of maize, showing the maximum development of tuberculate hairs. Compare with Plate LVI. Natural size.

PLATE LIX

A leaf sheath of a second-generation hybrid maize plant. This plant represents the maximum length of hairs. They are even longer than any thus far observed in the Esperanza variety. Compare with Plate LX. Natural size.

PLATE LX

A first-generation plant of Chinese×Esperanza maize hybrid. Measured by the number of sheaths with hairs, this was the most hairy plant in the first generation. Combined with this Esperanza character is an accentuation of the Chinese character of a scorpioid top.

PLATE LXI

A second-generation plant of a Chinese×Esperanza maize hybrid. This plant showed a maximum development of the Esperanza character of hairiness combined with the erect crowded leaf blades and deflexed tassel of the Chinese variety.

PLATE LXII

A second-generation plant of a Chinese×Esperanza maize hybrid. An extreme example of the scorpioid top; the angle was recorded as 190°.

PLATE LXIII

A second-generation plant of a maize hybrid, showing the "goose-neck" character that appeared for the first time in this hybrid. This plant showed few Esperanza characters. Although the plant is one-sided, it shows that the displacement of the tassel is not the result of crowding by the leaf blades.

COMPARATIVE STUDY OF THE AMOUNT OF FOOD EATEN BY PARASITIZED AND NONPARASITIZED LARVÆ OF CIRPHIS UNIPUNCTA

By DANIEL G. TOWER,

Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology

INTRODUCTION

The aim of an experiment which was conducted at the United States Entomological Laboratory in West La Fayette, Ind., during the summer of 1915, was to determine whether larvæ of the army worm (*Heliophila*) *Cirphis unipuncta* Haworth, when attacked by an internal parasite, *Apanteles militaris* Say, ate less, as much as, or were stimulated to eat more than when nonparasitized; and as a sequence, to determine whether this or a similar parasitism is directly beneficial in the generation parasitized or only indirectly, resulting in subsequent smaller generations. Although only 9 of the 25 parasitized larvæ with which the experiment was started lived until the emergence of the parasites, the others dying soon after oviposition took place, the records of these 9 larvæ are sufficiently definite to satisfy the purpose of the experiment.

The excellent work of Mr. J. J. Davis and Mr. A. F. Satterthwait [1] in determining the total amount of food eaten by healthy larvæ of *C. unipuncta* under different feeding conditions has been used to compare with the amount of food eaten by parasitized larvæ.

The results of the experiments have been drawn up in tabular form to show the life of the host larvæ from the time they were oviposited in until their death coincident with the emergence of the parasite and the life history of the parasite in relation to its host (Table I).[2]

EXPERIMENTAL METHODS

The parasites were induced to oviposit in the host larva while confined in test tubes into which a larva was introduced and left until recognized as a host and parasitized. Often this occurred immediately, and three or four ovipositions might take place before the larva could be removed. In other cases it would be some minutes before the parasite could be induced to oviposit.

These parasitized larvæ were confined separately in large vials, placed in the shade in a well-aired room, and fed pieces of mature corn leaves, conveniently cut out so as to measure 1 square inch each.

In order to obtain unfertilized females, individual cocoons were placed in gelatin medicine capsules previous to the emergence of the adults, the sex being easily determined through the transparent gelatin, when the adults emerged.

[1] Data as yet unpublished; may appear in a later issue of this Journal.

[2] The author was ably assisted in the care and feeding of the larvæ by Mr. H. J. Hart, who was temporary assistant at the laboratory during the summer of 1915.

TABLE I.—*Life-history data relating to host larvæ of Cirphis unipuncta and to the parasites, Apanteles militaris, attacking them*

Experiment No.	Width of head of host when oviposited in.	Age of host when oviposited in.	Date of ovipositions.	Number of ovipositions.	Duration of ovipositions in seconds.	Total amount of corn foliage eaten after parasitism.	Width of head of host when parasite emerged.	Age of host when parasite emerged.	Number of parasites in each host.	Date parasite spun cocoons.	Date adult parasite issued.	Time spent in host by parasite.	Time spent in cocoon by parasite.	Total life cycle of parasite.
	Mm.		1915.			*Sq. in.*	*Mm.*			1915.	1915.			
10	2.4	Fifth instar...	Aug. 15, 1.32 p. m	3+	1 each.....	16.21	3.4	Sixth instar.	49	Aug. 29, previous to 8 a. m.	Sept. 7, previous to 8 a. m.	13 d., 18 h., 28 m.	9 d.....	22 d., 18 h., 28 m.
11	2.1	.....do.....	Aug. 15, 1.37 p. m.	2+	1 or less each..	12.16	3	...do.....	39	Aug. 29, between 10.30 a. m. and 6 p. m.	Sept. 8, previous to 8 a. m.	13 d., 20 h., 53 m. to 14 d., 4 h., 23 m.	9 d., 21 h., 30 m. to 9 d., 14 h.	23 d., 18 h., 23 m.
15	2.1	.....do.....	Aug. 15, 1.50 p. m.	1	1.....	11.97	.....	...do.....	48	Aug. 29, previous to 8 a. m.	Sept. 7, between 8 and 11.30 a. m.	13 d., 18 h., 10 m.	9 d. to 9 d., 3 h., 30 m.	22 d., 18 h., 10 m. to 22 d., 21 h., 40 m.
16	2.2	.....do.....	Aug. 15, 2.03 p. m.	1+	1 each.....	14.50	3.2	...do	63	.....do	Sept. 7, previous to 8 a. m.	13 d., 17 h., 57 m.	9 d.....	22 d., 17 h., 57 m.
19	1.8	Fourth instar.	Aug. 15, 2.55 p. m.	2	.....do.....	20.63	3.5	...do.....	100	.....do.....	Sept. 8, previous to 8 a. m.	13 d., 17 h., 5 m.	10 d.....	23 d., 17 h., 5 m.
22	1.7	.....do.....	Aug. 15, 3.06 p. m.	3	About 2 each..	17.36	.....	...do.....	123	.....do.....	.....do.....	13 d., 16 h., 54 m.	10 d.....	23 d., 16 h., 54 m.
23	1.8	.....do.....	Aug. 15, 3.12 p. m.	3	.....do.....	21.41	3.6	...do.....	154	Aug. 31, previous to 7 a. m.	Sept. 8, between 8 and 10.30 a. m.	15 d., 15 h., 48 m.	8 d., 1 h. to 8 d., 3 h., 30 m.	23 d., 16 h., 48 m. to 23 d., 19 h., 18 m.
24	1.8	.....do.....	Aug. 15, 3.20 p. m.	3	.....do.....	17.64	3.4	...do.....	88	Aug. 29, previous to 8 a.m.	Sept. 8, previous to 8 a. m.	13 d., 16 h., 40 m.	10 d.....	23 d., 16 h., 40 m.
25	1.7	.....do.....	Aug. 15, 3.30 p. m.	3+	1+ each.....	17.99	3.3	...do.....	98	.....	Sept. 9, previous to 8 a. m.	.....	.....	24 d., 16 h., 30 m.
Average.....												14 d., 11½ m..	9 d., 8 h., 45 m.	23 d., 12 h., 26 m.

LIFE CYCLE OF THE PARASITE

The biology of *A. militaris* has already been studied and the results published.[1]

Oviposition took place with great rapidity and apparently anywhere in the host, attempts even being made by the parasite to oviposit in the head. The largest number of eggs inserted at one time, according to the observations herein recorded, was 154 for 3 ovipositions, averaging 51+ each (Table I, Experiment 23). The two endoparasitic stages and the egg stage required an average of 14 days, 11½ minutes, while the time spent by the third larval stage and the pupal stage in the cocoon averaged 9 days, 8 hours, and 45 minutes, and the average for the total life cycle was 23 days, 12 hours, and 26 minutes.

The parasitic larva leaves its host by means of an individual exit hole cut through the muscles and epidermis by its mandibles. As the larvæ squeeze through the holes they molt their second larval skins, and when about two-thirds of their way out commence to spin their cocoons. After the cocoon is spun and previous to pupation, the accumulated wastes are passed, being deposited at one end of the cocoon. Shortly following this the larva pupates and the last larval skin is pushed to the same end of the cocoon.

The adult issues, after kicking off its pupal skin, by cutting off a cap-like portion at one end of the cocoon, cleans itself, and at the same time passes a quantity of waste. It is now ready for copulation, oviposition, or feeding, as the case may be. In this respect it was found that females were at once ready to oviposit following emergence and previous to feeding or copulation, and that the progeny from such females were all males. Hence it is seen that unfertilized females give rise parthenogenetically to a generation of males.

CONCLUSIONS

In using the data compiled by Davis and Satterthwait on the amount of food eaten by healthy larvæ of *C. unipuncta*, for comparison with the amount eaten by parasitized larvæ, it will only be necessary to use the feeding records for the last three instars in one series of their experiments, this being the one in which the larvæ were confined in lantern-globe cages. These records were selected in preference to those obtained by keeping the larvæ in large vials, because in the former case a larger number of records were obtained, although in the latter case the averages of the feeding records for the same periods run higher.

Larvæ 10, 11, 15, and 16 were newly molted fifth-stage specimens when oviposited in, and they ate 16.21, 12.16, 11.97, and 14.50 square inches of corn foliage, respectively, during their last two stages previous to the emergence of the parasites, which is a much smaller amount than

[1] Tower, D. G. Biology of Apanteles militaris. *In* Jour. Agr. Research, v. 5, no. 12, p. 495–508, 1 fig., pl. 50. 1915.

the average of 33.6 square inches eaten by 20 nonparasitized larvæ during the same stages. Larvæ 19, 22, 23, 24, and 25 were partially developed fourth-stage specimens when oviposited in, and they ate, during the remainder of their life, which lasted until the parasites emerged from them some time during the last or sixth stage, 20.63, 17.36, 21.41, 17.64, and 17.99 square inches of corn leaf, respectively, as compared with the average of 34.77 square inches eaten by 20 nonparasitized larvæ during the last three stages. (See Table I.)

From these results it will be seen that parasitized larvæ ate approximately half as much as unparasitized larvæ during the same periods, and it seems conclusive, even from these few records, that parasitism by *A. militaris* is directly beneficial in the generation attacked. From the results obtained it might seem as though larvæ oviposited in at an earlier date would eat more before being killed, but the time spent in the host by the parasites seems to be fairly constant, and this was also noticed in a larger number of cases in former experiments with *A. militaris*. Hence, it is believed that in such cases the larvæ would have only approximately the same amount of time for feeding, and a larger portion of this period would occur during the earlier stages, when a much smaller amount of food is eaten, so that the amount eaten would be less than the normal for unparasitized larvæ.

ALEYRODIDAE, OR WHITE FLIES ATTACKING THE ORANGE, WITH DESCRIPTIONS OF THREE NEW SPECIES OF ECONOMIC IMPORTANCE

By A. L. QUAINTANCE, *Entomologist in Charge of Deciduous Fruit Insect Investigations*, and A. C. BAKER, *Entomological Assistant, Bureau of Entomology*

Thirteen species of so-called white flies are recorded in literature as infesting Citrus plants in different parts of the world. Eight of these are present in Florida, four of them being native to the United States and four having been introduced. The native forms have thus far been of little economic importance, whereas two of the introduced species are first-class Citrus pests. The remaining two introduced forms, although recently established on the orange (*Citrus aurantiaca*), have already attracted attention by reason of their injuries. Our knowledge of the remaining five species of Citrus white flies, while meager, indicates that these, in their range of distribution, are abundant and destructive and would in all probability prove to be very undesirable immigrants. The new forms treated herein must be classed in the same category, especially *Aleurocanthus woglumi*, which, although previously named, is here technically described for the first time. This last species, of oriental origin, has already found its way to Jamaica and the Bahamas, where it infests the orange to a serious extent.

The present paper brings together the essential information concerning the distribution and food plants of the white flies which attack Citrus plants and describes three new species of economic importance.

Aleurocanthus citricolus (Newstead)

Aleurodes citricola Newst., 1911, *in* Mitt. Zool. Mus. Berlin, Bd. 5, Heft 2, p. 173.[1]

This species is known only from the original description. It was taken at Dar es Salaam, German East Africa, on *Citrus* sp. in 1902. The immature stages occurred in large, overcrowded colonies, appearing to the unaided eye as patches of a sootlike deposit on the lower surface of the leaves. This is one of the spiny forms and bears a general resemblance to *A. woglumi* (fig. 2, *A–J*, Pl. LXIV, LXV).

Aleurocanthus citriperdus, n. sp.

This insect (fig. 1) was taken by Mr. R. S. Woglum, of the Bureau of Entomology, in several localities in the Orient, as follows: Royal Botanic Gardens, Ceylon, on an unknown tree, October, 1910; Lahore, India, on *Citrus* sp., July, 1911; Buitenzorg, Java, on orange, January, 1911; Sandan Glaya, Java, on *Citrus* sp., January, 1911. It is reported

[1] All bibliographic citations in synonymy are given in full in "Literature cited," pp. 471–472.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.
cl

Vol. VI, No. 12
June 19, 1916
K—37

37769°—16——3

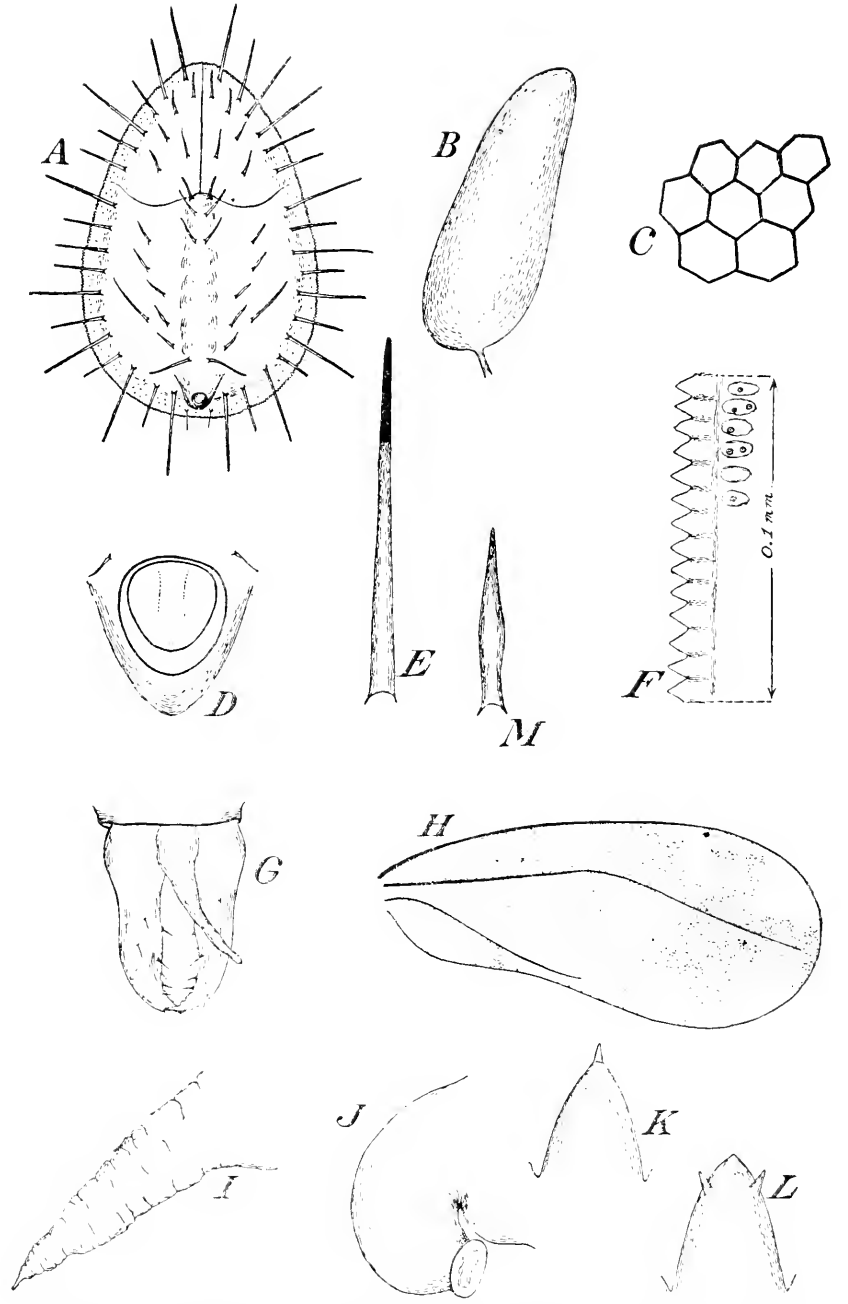


FIG. 1.—*Aleurocanthus citriperdus*: *A*, Pupa case; *B*, egg; *C*, polygonal markings of egg; *D*, vasiform orifice of pupa case; *E*, spine from dorsum of pupa case; *F*, margin of pupa case; *G*, genitalia of adult male; *H*, forewing of male; *I*, antenna of pupa case; *J*, leg of pupa case; *K*, *L*, marginal teeth, much enlarged; *M*, central swollen spine from dorsal area.

as occurring abundantly on species of Citrus and is regarded as of considerable economic importance.

PUPA CASE (fig. 1, *A*).—Length 1.36 mm.; width 0.96 mm.; shape elliptical to oval, broadest across the third abdominal segment, narrowest cephalad. Dorsum with a moderate central abdominal ridge on which the abdominal segments are not distinctly marked off, though they may be distinguished. Submarginal area somewhat flat; suture separating the thorax and abdomen quite distinct; surface appearing somewhat granular or faintly corrugated, an appearance which may be due to difference in pigmentation. Dorsum with numerous heavy spines (fig. 1, *E*) which after clearing remain black at the tips, but are otherwise a clear greenish yellow. These are arranged as follows: On the submarginal area is a more or less even row of usually 32 spines. This row is composed of two series alternating with one another. The one is made up of spines averaging about 0.288 mm., and the other of spines averaging 0.192 mm. in length. Near the medio-dorsal abdominal line there are three pairs of spines, one pair situated about 0.19 mm. anterior to the vasiform orifice and the others on the cephalic part of the abdominal region. The spines of the pair on the first abdominal segment are somewhat more widely separated than those of the other two pairs. Six other pairs of spines are present on the abdomen. Five of these pairs are short, about 0.08 mm. long, and form an even subdorsal row on each side, the rows thus formed diverging on the cephalic part of the abdomen. The remaining pair is composed of much longer spines, situated about 0.29 mm. from the thoracic suture and about the same distance from the lateral margin of the case. On the thorax there is a subdorsal row of four spines on each side (fig. 1, *M*) and near the medio-dorsal line another pair of spines is present. Just anterior to the vasiform orifice a pair of tubercled setæ is situated, and another pair is present on the medio-caudal portion of case. The margin of the case (fig. 1, *F*) is dentate, the teeth (fig. 1, *K*, *L*) being rather fine and acute. A distance of 0.16 mm. is occupied by twelve of the teeth. At the base of the teeth small clear areas are found, and some distance in from the margin a row of elliptical areas, possibly glands, are present. These appear to be on the under surface of the case, while on the submarginal dorsal region, scattered between the margin and the insertion of the spines, are small dark pores. The vasiform orifice is situated on a tubercle which forms the caudal portion of the medio-dorsal ridge. It is subcircular in outline, tending to cordate. The operculum is somewhat similar in shape and obscures the lingula. The color of cleared specimens under the microscope is a light brown, with the margin and the borders of the dorsal ridge darker.

On the leaf the cases are shining black. There is little or no dorsal secretion, but a short, white, waxy marginal fringe is present. The rods forming this fringe are not distinct, but are more or less frayed and give a woolly appearance to the outer edge of the fringe. In some specimens, however, this woolly appearance is not evident, but the wax forms a series of marginal plates. When the pupæ are removed from the leaf, their former position is marked by the white oval wax ring which remains attached to the leaf. The larvæ present a similar appearance on the leaf, but are brown instead of black.

ADULT MALE.—Length 0.96 mm.; general color brownish, shaded with dusky. Vertex rounded, with a longitudinal median ridge, color dark brown; ocelli clear; compound eyes Vandyke, constricted; antennæ absent in the specimens at hand; labium tipped with dusky; thorax shaded with dusky. Forewings 0.88 mm. long by 0.35 mm. wide, marked with dark bluish gray, as indicated in fig. 1, *H*. Veins olive color; radial sector bent caudad at 0.4 mm. from the distal end. Hind wings 0.64 mm. long and 0.25 mm. wide at widest part; color uniform dusky, vein olive color. Legs with the femora and the proximal half of the tibiæ dusky, the remainder of the tibiæ and the tarsi greenish yellow. Fore femora 0.19 mm.; fore tibiæ 0.23 mm.; fore tarsi, proximal segment 0.08 mm., distal 0.064 mm.; middle femora 0.24 mm.,

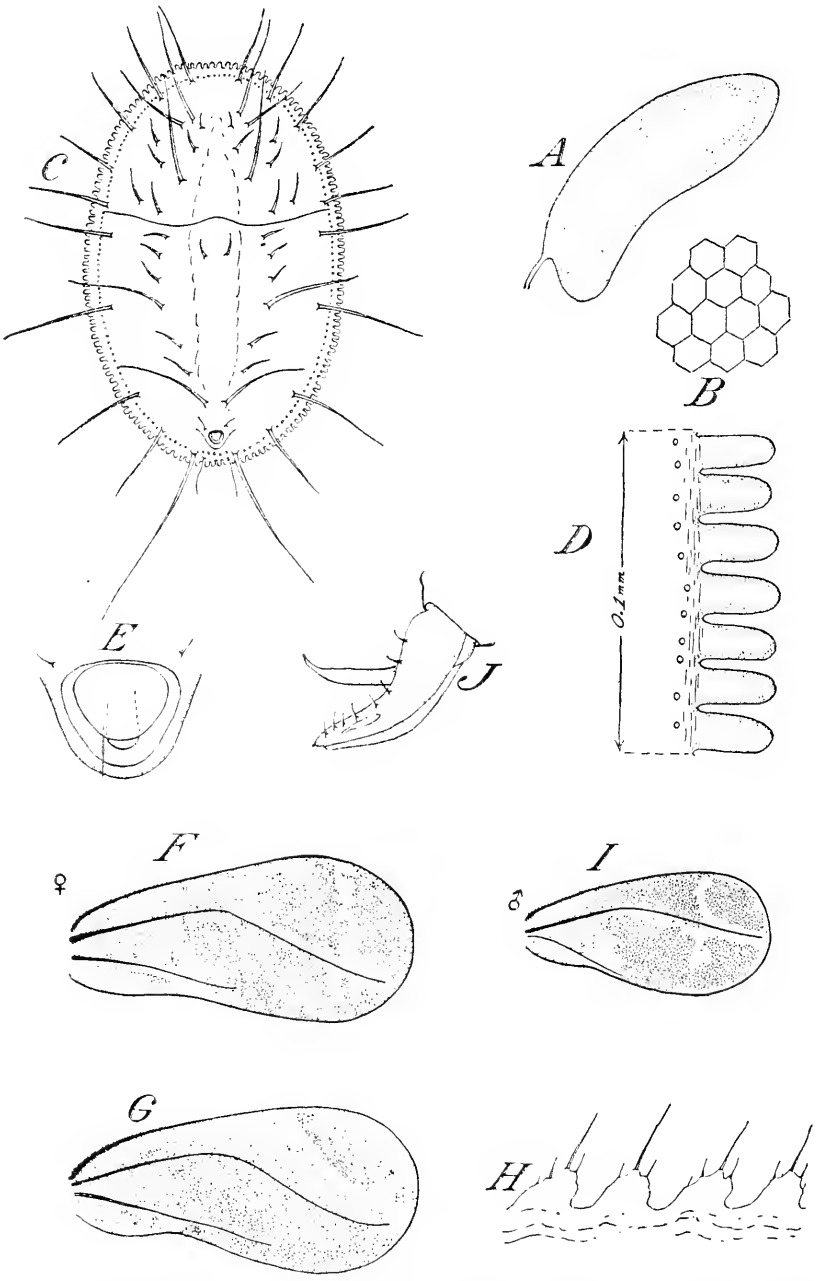


FIG. 2.—*Aleurocanthus woglumi:* *A*, egg; *B*, polygonal markings of egg; *C*, pupa case; *D*, margin of pupa case; *E*, vasiform orifice of pupa case; *F*, forewing of adult female; *G*, same, showing variation in markings; *H*, costal margin at base of wing of female; *I*, forewing of male; *J*, male genitalia.

hind tibiæ 0.36 mm., hind tarsus, proximal 0.112 mm., distal 0.72 mm. Claws normal, with a hairy central paronychium; genital segment dark brown, 0.112 mm., broad at the insertion of the claspers. These latter are dark-brown, becoming lighter at their distal tips. They are 0.128 mm. long and each about 0.03 mm. at the shoulder near the base. They are acute at the tips, curved inward, and armed on the inner margin with a number of prominent spines (fig. 1, *G*). A few small hairs are scattered here and there, situated on small tubercles. The penis is as long as the claspers, somewhat bulbous at the base, greenish yellow, and slightly curved upward.

ADULT FEMALE.—Unknown.

Described from adult males in balsam mounts and numerous pupa cases in balsam mounts and dry upon the foliage.

Type.—Cat. No. 19099, U. S. National Museum.

Aleurocanthus woglumi Ashby.[1]

Aleurocanthus woglumi Quain., Ashby, 1915, *in* Ann. Rpt. Dept. Agr. Jamaica, 1914/15, p. 31.
Aleurocanthus woglumi Quain., Ashby, 1915, *in* Bul. Dept. Agr. Jamaica, n. s. v. 2, no. 8, p. 322.

Specimens of this species (fig. 2; Pl. LXIV, LXV), which may be called the "spiny Citrus white fly," were first received by the Bureau of Entomology on June 16, 1910, from Dr. E. W. Berger, the material coming from India from H. Maxwell-Lefroy. Specimens were also received in 1910 from Mr. George Compere, who had collected the insect in the Philippine Islands. During 1910 and 1911 Mr. R. S. Woglum, in the course of his search for parasites of the orange white fly (*Dialeurodes citri* Ashm.), found this insect common and widely distributed on orange in India and Ceylon, and it has subsequently been received from that region from Mr. A. Rutherford.

Our first knowledge of its presence in the Western Hemisphere came with the receipt of specimens from Col. C. Kitchener, Half Way (Kingston), Jamaica, on November 27, 1913. Additional material was received during 1914 from Jamaica from Col. Kitchener and from Prof. S. F. Ashby, Microbiologist of the Jamaica Department of Agriculture. Under date of February 5, 1916, specimens were submitted by Mr. P. Cardin, Entomologist of the Cuba Agricultural Experiment Station, for verification of determination made by Prof. Ashby. On February 7, 1916, a large lot of orange leaves infested with *A. woglumi* was received from Mr. L. J. K. Brace, Nassau, New Province, Bahamas, who states:

> Certain orchards in this island at least have been very much affected with this insect, all of the leaves being so much infested on their undersurfaces that they present a black appearance, not only killing the trees but causing some persons to attempt to stop the mischief by cutting down the trees, though the young shoots become again covered * * *. I have no doubt that the planters' exchange have introduced this pest from the East. Plants have been for some time obtained by individuals here from the Jamaican establishment and also from Florida.

Prof. Ashby thinks the insect was introduced into Jamaica on the mango during the last 20 years. In that island it has become very

[1] *Aleurocanthus woglumi*, the writers' manuscript name for this species, was furnished to Prof. Ashby. According to the International Code, his descriptive remarks, as cited, make him the author of the species.

prominent, infesting the leaves of all species of Citrus on the lowland plains. Honeydew is excreted in small amounts, which is followed by the development of sooty fungi, but not to the extent that is true of certain other white flies and scale insects.

The present known distribution and food plants are shown in Table I.

TABLE I.—*Present known distribution and food plants of Aleurocanthus woglumi*

Date.	Quaintance No.	Locality.	Host plant.	Collector.
June 16, 1910.	5264	India	Orange	Maxwell-Lefroy.
1910	6763	Manila, P. I	do	George Compere.
1910	6764	do	do	Do.
Oct., 1910	6750	Royal Bot. Gardens, Ceylon.	*Capparis roxburghi*	R. S. Woglum.
Do	6744	do	*Capparis pedunculosus*	Do.
(?)	6553	India	Unknown tree	Do.
Nov., 1910	6556	Lahore, India	*Citrus* sp	Do.
Do	6564	Gujranwala, India	do	Do.
Do	6557	Lahore, India	do	Do.
Do	6560	do	do	Do.
Dec., 1910	6528	Kalimpong, Sikkim, India.	do	Do.
June, 1911	8021	Lahore, India	*Citrus* sp. and *Morus* sp	Do.
Sept., 1911	8012	Nagpur, C. P., India	(?)	Do.
Aug., 1913	8753	Peradeniya, Ceylon	*Salacia reticulata*	A. Rutherford.
Sept., 1913	8768	do	*Kurrimia zeylanica*	Do.
Nov., 1913	8748	Half Way, Jamaica	Orange	Col. C. Kitchener.
Feb., 1914	8782	do	do	Do.
May, 1914	8922	Kingston, Jamaica	*Citrus* sp.; *Guiacum officinale; Cestrum nocturnum* L.	S. F. Ashby.
Feb., 1916	12066	Guantanamo, Cuba	Orange	P. Cardin.
Do	12067	Nassau, N. P., Bahama	do	L. J. K. Brace.

EGG (fig. 2, *A*).—Size, 0.208 mm. by 0.08 mm.; shape elliptical, curved, with the stalk short and attached some distance from the base. Color yellowish, surface apparently without reticulations in some cases and with them in others, which is no doubt due to the structure being destroyed in boiling. When they are present (fig. 2, *B*) they average 0.006 mm. in diameter.

LARVA.—Larvæ are present in the material at hand, but they are in too poor a condition for accurate description. They are brown in color and armed with numerous long spines.

PUPA CASE (fig. 2, *C*).—Size variable in the different lots of material, averaging 1.4 by 0.89 mm.; shape regularly elliptical, with the dorsum considerably arched or rounded; the median ridge high, but not markedly distinct from the dorsal area, excepting near the caudal portion of the abdomen and at the vasiform orifice, which is elevated into a more or less prominent tubercle. Color dense black, so much so that it is almost impossible, even after prolonged boiling, to make out details. When the denser dorsal portion of the case is removed the ventral part appears under the microscope as dark brown and more or less irregularly mottled. Submarginal area with usually 20 spines forming a ring. These vary consideably in length, but the caudal pair is nearly always the longest. The spines are curved outward. A pair of hairs is present on the caudal margin caudad of the vasiform orifice. The spines on the dorsum are small excepting two pairs on the abdomen and three pairs on the thorax. Their number and arrangement are shown in the figure. The vasiform orifice (fig. 2, *E*) is prominent, being on a tubercle, but is small. It is somewhat triangular in shape, tending to circular. The operculum almost entirely fills the orifice obscuring the lingula—all but a very small portion at the tip. Cephalad of the orifice a pair of minute setæ is situated one on each side. The margin of the case

is dentate, the teeth large and bluntly rounded (fig. 2, *D*). The inner spaces are not acute, but often squarely truncate. A space of 0.1 mm. is occupied by six or seven teeth. On this feature alone the case is easily separable from those of the other species. At the base of the teeth, forming a ring around the case, is a series of minute, clear, porelike areas. On the leaf the case is jet black with the dorsum somewhat arched and the abdominal segments marked, but not distinctly separated. On the margin all around is a narrow cottony lateral wax fringe. This sometimes extends mesad, irregularly covering the submarginal area, but dorsal secretion is usually absent.

ADULT FEMALE.—Length from vertex to tip of ovipositor, 1.12 mm.; color brown, under the microscope a deep wine color with darker shadings on head, thorax, and tip of abdomen. The specimens at hand are somewhat imperfect and it is difficult to make out the structure. The vertex seems to be rounded and possessed of a slight median ridge. The eyes are very dark brown. The antennæ are absent from the specimens at hand. Labium yellowish, tipped with black. Legs yellowish, shaded on femora with dusky. The femora and tibiæ of the hind legs are considerably darker than the others; length of hind femora 0.288 mm.; hind tibiæ 0.432 mm. The tarsi have the proximal segment 0.1 mm. and the distal 0.06 mm. The proximal segment is armed on its distal extremity with one large spine and several smaller ones; the foot is normal, with the paronychium straight and hairy. The forewings (figs. 2, *F*, *G*) are 1.268 mm. long and 0.76 mm. wide at the widest part. The radial sector is heavy, yellowish brown in color, and much curved. The cubitus is very fine, long and slightly curved, that portion of the wing below it forming a more or less distinct lobe. In color the wing is a deep smoky, excepting as follows: A line following the cubitus, and a rather large spot near its distal extremity are colorless. A line following the radial sector from its distal extremity to almost its median curve, and another crossing it almost at right angles are colorless. This gives the appearance of a white cross on a dark background. In some wings the marking is not so evident, but there is one curved colorless line angling across the wing a short distance above and parallel with the radial sector. The border of this white line seems more heavily shaded than the remainder of the wing. The margin of the wing (fig. 2, *H*) is armed with a series of rather prominent teeth directed toward the distal extremity of the wing. Each one of these is armed with one prominent spine and usually three smaller ones. The margin formed by these teeth and a line along their bases is bright wine red. The hind wing is uniform smoky, with the vein yellowish brown.

ADULT MALE.—Much smaller than the female, measuring only about 0.79 mm. from vertex to tip of claspers. The specimens are in poor condition, the antennæ are absent, and it is impossible to make out the structure with certainty. The color is a yellowish or a reddish brown. The hind femora, 0.24 mm. and the hind tibia, 0.4 mm. in length. They are marked as in the female. The claspers (fig. 2, *J*) are 0.126 mm. long. Near their distal ends there are a number of jagged teeth and they are armed with a number of long slightly curved hairs, those near the tip being the longest. The penis is as long as the claspers, yellowish, and almost straight.

Described from females, males, and pupa cases in balsam mounts and pupa cases and eggs on the leaves.

Aleurocanthus spiniferus (Quaintance)

Aleurodes spinifera Quain., 1903, *in* Canad. Ent., v. 35, no. 3, p. 63.

Collected on *Citrus* sp. and rose by Mr. C. L. Marlatt, of the Bureau of Entomology, at Garalt, Java, on December 7, 1901; also taken on orange at Macao, South China, by Mr. R. S. Woglum, in February, 1911.

Aleurolobus marlatti (Quaintance)

Aleurodes marlatti Quain., 1903, in Canad. Ent., v. 35, no. 3, p. 61.

This species (Pl. LXVI, fig. 3) was collected by Mr. C. L. Marlatt on May 17, 1901, at Kumomoto, Japan, on orange; also by Mr. R. S. Woglum on *Citrus* sp. and *Morus* sp. at Lahore, India; also collected by Mr. Woglum on *Ficus* sp. in the Royal Botanic Gardens, Ceylon; on an unknown tree in the Botanic Gardens, Buitenzorg, Java. This insect has also been received by the Bureau of Entomology from Mr. S. I. Kuwana, collected at Fukuoka, Japan. Mr. Kuwana states that this same species has been collected on Rivkin Island. One lot of infested orange leaves is also in the Bureau collection from Tokyo, Japan.

Aleurothrixus floccosus (Maskell)

Aleurodes floccosa Mask., 1896, *in* Trans. and Proc. N. Zeal. Inst., v. 28 (n. s. v. 11), 1895, p. 432.
Aleurodes horridus Hempel, 1899, *in* Psyche, v. 8, no. 280, p. 394.

This species (fig. 3, *H*) is based on material from Jamaica on lignum-vitæ (*Guaiacum officinale*?) and was first recorded on orange by Cockerell (1902) [1] from Mexico. The insect has several color phases, ranging from clear yellow, the typical and more abundant form, to individuals with the dorsum striped with dark brown, or with the dorsal disk dark brown and the submarginal area yellow, etc.

Hempel's *A. horridus* from Brazil on guava (*Psidium guajava*) is apparently the same as *A. floccosus*. This latter differs from *A. howardi* only in the absence of a comb of teeth on the caudal margin of the vasiform orifice (fig. 3, *H*). Both *A. floccosus* and *A. howardi* are almost always present together on the same leaf and their food plants and distribution are practically identical. *A. floccosus* is common in the islands of the West Indies and also occurs in Florida, Mexico, British Guiana, Brazil, Argentina, Canal Zone, Chile, and Paraguay. In addition to the orange, lime, grapefruit, etc., *A. floccosus* has been taken on the sea-grape (*Coccoloba uvifera*), *Plumeria* sp., *Baccharis genistelloides*, guava, a coarse grass, and a climbing vine.

Aleurothrixus howardi (Quaintance)

Aleyrodes howardi Quain., 1907, U. S. Dept. Agr. Bur. Ent. [Bul.] 12, pt. 5, Tech. Ser., p. 91.

This species (fig. 3, *E*, *J*; Pl. LXVII) occurs on the same host plant and has the same distribution as *A. floccosus*. It was apparently first found in Florida by Prof. P. H. Rolfs at Miami on sea-grape, September 25, 1900, and therefore gained a foothold in that State some years previous to its discovery by Dr. E. A. Back.

Aleurothrixus porteri, n. sp.

This species (fig. 3, *A–D*, *F*, *G*, *I*, *K*, *L*; Pl. LXVIII) has been received only from Chile and Brazil. The first collection was sent by Prof. T. D. A.

[1] Bibliographic citations in parentheses refer to "Literature cited," pp. 471–472.

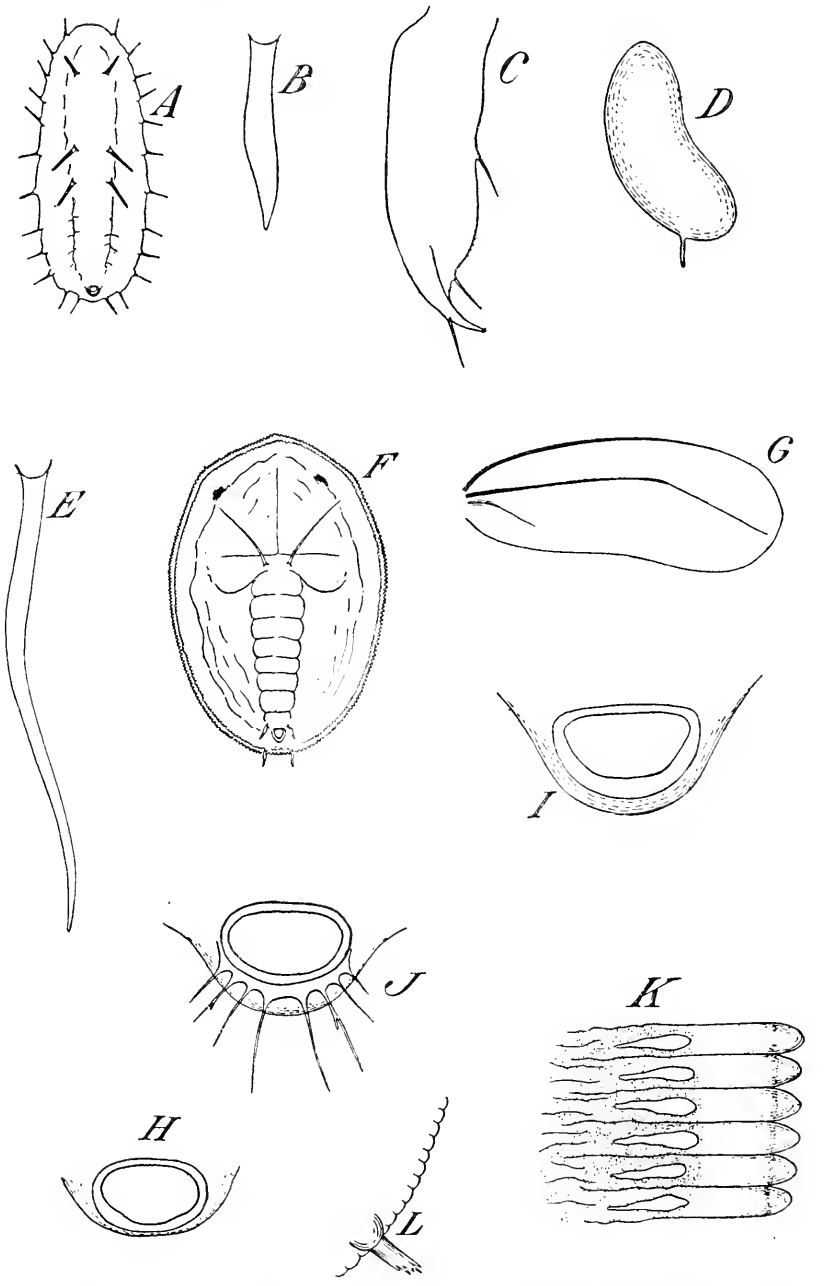


FIG. 3.—*Aleurothrixus porteri, A. howardi,* and *A. floccosus: A, Aleurothrixus porteri:* Larva, first instar. *B, A. porteri:* Caudal spine of pupa case. *C, A. porteri:* Clasper of male. *D, A. porteri:* Egg. *E, A. howardi:* Caudal spine. *F, A. porteri:* Pupa case. *G, A. porteri:* Forewing of adult. *H, A. floccosus:* Vasiform orifice of pupa case. *I, A. porteri:* Vasiform orifice of pupa case. *J, A. howardi:* Vasiform orifice of pupa case. *K, A. porteri:* Margin of pupa case. *L, A. porteri:* Margin of early larva.

Cockerell on June 7, 1895, who received the material from Mr. Lataste, under the name *phalaenoides*.

In a letter to the senior author in January, 1905, Cockerell suggested that Lataste supposed the species to be Blanchard's *phalaenoides*. Since that time we have shown that *phalaënoides* Blanchard is a species of Aleurodicus. Table II records the distribution and food plants of the specimens of *A. porteri* in the collection of the Bureau of Entomology.

TABLE II.—*Distribution and food plants of Aleurothrixus porteri in the collection of the Bureau of Entomology*

Date.	Collector.	Host.	Bureau No.	Locality.
Feb. 13, 1894..	M. Lataste.....	Orange.....	Q. 4062....	Santiago, Chile.
May 14, 1894..	.....do.....	.....do.....	Q. 4063....	Do.
Mar. 14, 1895..	.....do.....	(?).....	Q. 4064....	Chile.
Feb. 4, 1896...	Edward Reed.....	Orange.....	Q. 4065....	Ransagua, Chile.
Apr. 1, 1899...	D. G. Fairchild.....	Solanaceous plant.....	Q. 351.....	Villa del Mar, Chile.
Oct., 1904....	M. J. Rivera.....	Orange?.....	Q. 3214....	San Bernardo, Chile.
Oct. 25, 1904..	.....do.....	*Schinus dependens* Ortega.	Q. 12022...	Santiago, Chile.
June 20, 1912.	Prof. Carlos E. Porter..	*Schinus molle*.....	Q. 8726....	Chile.
Mar., 1913....	....do.....	Orange.....	Q. 8820....	Santiago, Chile.
Jan. 5, 1914...	Popenoe and Dorsett....	Jaboticaba.....	Q. 12004...	Rio de Janeiro, Brazil.
(?).....	Prof. Carlos E. Porter...	*Lippia citriodora* Kunth.	Q. 12024...	Santiago, Chile.
Aug. 8, 1915..	.....do.....	Myrtus.....	Q. 12062...	Do.

Of this material, Quaintance No. 351 is chosen for the type.

LARVA, FIRST STAGE (fig. 3, *A*).—Size 0.352 by 0.208 mm. Shape elongate elliptical; abdomen with a moderately distinct keel, the caudal extremity of which projects to the vasiform orifice; dorsum armed with four pairs of stout straight spines; margin very minutely serrate and armed on its caudal part with a pair of long curved spines and the remainder of the margin with 11 pairs of minute spines; antennæ straight, not quite as thick as the dorsal spines and extending slightly beyond the margin; vasiform orifice almost completely filled by the operculum; color under the microscope pale brown.

PUPA CASE.—Size 0.88 by 0.502 mm.; shape elliptic, some specimens slightly broader across the thorax than across the abdomen; dorsum somewhat elevated, the abdomen with a distinct keel; incisions between marginal wax tubes shallow; vasiform orifice (fig. 3, *I*) small, elevated, operculum filling about half of the orifice and obscuring the lingula; spines latero-cephalad of the vasiform orifice and those on the caudal margin of case short, stout, and somewhat vasiform (fig. 3, *B*); those on the medio dorsum long; other characters very similar to those of *A. floccosus*. Color varying from yellow to dark brown and with flocculent wax as in *A. floccosus*.

ADULT MALE.—Color yellow, eyes dark brown; clasper rather short (fig. 3, *C*), its spur acute and not armed within with lobes; a few prominent spines present; length 0.08 mm.; length of insect from vertex to tip of claspers 0.88 mm.; forewing 1.04 mm. long, without markings, but often uniformly clouded with dusky.

ADULT FEMALE.—Similar to male in color; length 1.12 mm.; forewing 1.28 mm.

The adults in the collection are poorly preserved, and it is impossible to describe them in detail.

Described from larvæ, pupa cases, and adults in balsam mounts and pupa cases upon foliage.

Type.—Cat. No. 20171, U. S. National Museum.

Bemisia giffardi (Kotinsky)

Aleyrodes giffardi Kotin., 1907, *in* Bd. Com. Agr. and Forest. Hawaii Div. Ent. Bul. 2, p. 94.

This insect is reported present on Citrus trees in several gardens in Honolulu, where it is stated to be so abundant that the foliage of the trees becomes blackened by the sooty fungus growing on the exuded honeydew. Mr. Kotinsky believes that the insect has been introduced into Hawaii, and this opinion is strengthened by its discovery in collections of material made by Mr. Woglum at Lahore, India, in 1911. The host, however, was an unknown tree.

Dialeurodes citri (Ashmead) [1]

Aleyrodes citri Riley and Howard, 1893, *in* Insect Life, v. 5, no. 4, p. 219.
Aleurodes eugeniae, var. *aurantii* Mask., 1896, *in* Trans. and Proc. N. Zeal. Inst., v. 28 (n. s. v. 11), 1895, p. 431.
Aleyrodes aurantii Ckll., 1903, *in* Fla. Agr. Exp. Sta. Bul. 67, p. 665.

This is the destructive Citrus white fly of Florida, where it has been known since about 1880 (Pl. LXVI, fig. 1). It is rather generally distributed over the orange-growing regions of the Gulf States and is common on chinaberry and Cape jasmine considerably north of the Citrus belt. It is also recorded from Colorado, Illinois, and the District of Columbia, where it is probably confined to conservatories. This insect was discovered in California in 1907 and serious attempts were made to effect its eradication. It is still present in one locality (Marysville), where it is now so widespread and abundant that its eradication is considered to be impracticable (Weldon, 1915).

Dialeurodes citri is undoubtedly of oriental origin. It has been received from numerous localities in India, Ceylon, Japan, China, etc. According to Kirkaldy it is present in Chile, Mexico, and Brazil. In addition to Citrus plants, the insect in Florida infests numerous others as *Melia azedcrach*, *Gardenia jasminoides*, *Ligustrum* spp., *Diospyros kaki*, *Diospyros virginiana*, *Syringa* sp., *Coffea arabica*, *Ficus nitida*, etc. This and nearly related species are very generally parasitized in the Orient by certain four-winged flies, which are in that region apparently effective checks on their undue increase.

Dialeurodes citrifolii (Morgan)

Aleyrodes citrifolii Morgan, 1893, La. Agr. Exp. Sta. Spec. Bul., p. 70.
Aleyrodes nubifera Berger, 1909, Fla. Agr. Exp. Sta. Bul. 97, p. 67.
Aleyrodes nubifera Mor. and Back, 1911, U. S. Dept. Agr. Bur. Ent. Bul. 92, p. 86.

This species, long confused with *D. citri*, may be readily distinguished from that species by the reticulate eggs, character of the tracheal folds of the pupa case, and the smoky patch on front wings of the adults. The insect is known from North Carolina, Mississippi, Louisiana, California,

[1] This species was first fully described by Riley and Howard in Insect Life, as cited, but had earlier been named and briefly described in The Florida Dispatch, November, 1885, by W. H. Ashmead, who, according to the rules of the International Code, must be known as the author of the species.

and Florida. While not as important as *D. citri*, it is nevertheless decidedly noxious. It is also known to occur in Cuba and Mexico. No specimens of this insect were found in the Woglum collection of white flies from India, Ceylon, and other points in the East visited by him. By reason of its affinities, *D. citrifolii* is, however, almost surely oriental in origin.

This species, with one exception, is known to attack only Citrus plants. It was found on *Ficus nitida* growing in greenhouses at Audubon Park, New Orleans, La.

Paraleyrodes perseae (Quaintance)

Aleurodes perseae Quain., 1900, U. S. Dept. Agr. Div. Ent. [Bul.] 8, Tech. Ser., p. 32.
Paraleyrodes perseae Quain. and Baker, 1913, U. S. Dept. Agr. Bur. Ent. [Bul.] 27, pt. 1, Tech. Ser., p. 82,

This species is known only from Florida, where it is frequently found on orange, though never in destructive numbers thus far. It also feeds upon *Persea*, the avocado (*Persea americana*), and doubtfully on persimmon (*Diospyros* spp.). Several species of the genus are common in the West Indies, *perseae* being the only one known from the United States.

Trialeurodes floridensis (Quaintance)

Aleurodes floridensis Quain., 1900, U. S. Dept. Agr. Bur. Ent. [Bul.] 8, Tech. Ser., p. 26.

T. floridensis has thus far been recorded by the Bureau of Entomology only from Florida, where it is rather generally distributed. It infests avocado, guava, *Annona squamosa*, and the orange. While often very numerous on guava and avocado, it is at present of no importance on orange.

Trialeurodes vitrinellus (Cockerell)

Aleyrodes vitrinellus Ckll., 1903, *in* Ent. News, v. 14, no. 7, p. 241.

The type of this species is from Mexico on orange. Apparently the same insect has been taken in southern California on oak. Its injuries to orange in Mexico are probably not great.

Tetraleurodes mori (Quaintance)

Aleurodes mori Quain., 1899, *in* Canad. Ent., v. 31, no. 1, p. 1.

This indigenous species (Pl. LXIX, fig. 2) is widely distributed over the eastern United States and occurs on a large variety of plants, as mulberry, sycamore, maple, dogwood, hackberry, persimmon, holly, mountain laurel, etc. It has been found several times on orange, but not as yet in injurious numbers. That it may become troublesome under certain conditions, however, will be evident from the discussion relative to *T. mori*, var. *arizonensis*, which follows:

Tetraleurodes mori, var. **arizonensis** (Cockerell)

Aleyrodes mori, var. *arizonensis* Ckll., 1903, *in* Fla. Agr. Exp. Sta. Bul. 67, p. 666.
Aleurodes mori Ckll., 1900, *in* Sci. Gossip, n. s. v. 6, no. 72, p. 366.

Described from specimens taken in Arizona on orange (Pl. LXIX). The variety *T. mori arizonensis* is stated to differ from the typical *T. mori* in having the wings white marked with black without any red. An examination of the type specimens after mounting shows the presence of red markings on wings exactly as in *T. mori*, and we are unable to distinguish any characters in support of its status as a variety. On different occasions the Bureau of Entomology has received from Mexico an aleyrodid seriously infesting the orange (Pl. LXIX) which we are unable to distinguish in the immature stages from *T. mori*, and this species is considered by Cockerell to be identical with his variety *T. mori arizonensis*. While the variety, in our judgment, is invalid, we retain the name to designate a race of *T. mori* which, in Mexico, for some reason breeds abundantly on orange and is a pest of importance. *T. mori arizonensis* occurs only on orange in Mexico so far as bureau records indicate. It was first collected in 1894 by Dr. C. H. T. Townsend at Guadalajara and San Luis, and again by Townsend in 1902 at Zapotlan. Two lots of material were received from Prof. A. L. Herrera in 1905, without statement as to locality.

LITERATURE CITED

ASHBY, S. F.
1915. Notes on diseases of cultivated crops observed in 1913–14. Black fly or "black scale." *In* Bul. Dept. Agr. Jamaica, n. s. v. 2, no. 8, p. 321–322.

———
1915. Report of the microbiologist. Citrus diseases. *In* Ann. Rpt. Dept. Agr. Jamaica, 1914–15, p. 31.

BERGER, E. W.
1909. White-fly studies in 1908. Fla. Agr. Exp. Sta. Bul. 97, p. 39–71, 19 fig.

COCKERELL, T. D. A.
1900. Economic entomology in Arizona. *In* Sci. Gossip, n. s. v. 6, no. 72, p. 366–367.

———
1902. A synopsis of the Aleyrodidæ of Mexico. *In* Mem. y Rev. Soc. Cient. "Antonio Alzate," t. 18, p. 203–208, 3 fig.

———
1903. Aleyrodes (Trialeurodes) vitrinellus Ckll. *In* Ent. News, v. 14, no. 7, p. 241.

———
1903. White fly. (Aleyrodes citri) and its allies. *In* Fla. Agr. Exp. Sta. Bul. 67, p. 662–666.

HEMPEL, Adolph.
1899. Descriptions of three new species of Aleurodidae from Brazil. *In* Psyche, v. 8, no. 280, p. 394–395.

KOTINSKY, Jacob.
1907. Aleyrodidae of Hawaii and Fiji, with descriptions of new species. *In* Bd. Com. Agr. and Forest. Hawaii, Div. Ent. Bul. 2, p. 93–103, pl. 1.

MASKELL, W. M.
1896. Contributions toward a monograph of the Aleurodidæ, a family of Hemiptera-Homoptera. *In* Trans. and Proc. N. Zeal. Inst., v. 28 (n. s. v. 11), 1895, p. 411–448, pl. 24–35.

MORGAN, H. A.

1893. The orange and other citrus fruits, from seed to market, with insects beneficial and injurious, with remedies for the latter. La. Agr. Exp. Sta. Spec. Bul. 110, p. 36, fig., 3 pl. (2 col.).

MORRILL, A. W., and BACK, E. A.

1911. White flies injurious to citrus in Florida. U. S. Dept. Agr. Bur. Ent. Bul. 92, 109 p., 19 fig., 10 pl. (1 col.).

NEWSTEAD, Robert.

1911. On a collection of Coccidae and Aleurodidae, chiefly African, in the collection of the Berlin Zoological Museum. *In* Mitt. Zool. Mus. Berlin, Bd. 5, Heft 2, p. 155–174.

QUAINTANCE, A. L.

1899. New, or little known, Aleurodidæ–I. *In* Canad. Ent., v. 31, no. 1, p. 1–4, illus.

———

1900. Contributions toward a monograph of the American Aleurodidæ. *In* U. S. Dept. Agr. Div. Ent. [Bul.] 8, Tech. Ser., p. 1–64, 8 pl.

———

1903. New oriental Aleurodidæ. *In* Canad. Ent., v. 35, no. 3, p. 61–64.

———

1907. The more important Aleyrodidae infesting economic plants, with description of a new species infesting the orange. U. S. Dept. Agr. Bur. Ent. [Bul.] 12, pt. 5, Tech. Ser., p. 89–94, fig. 23–24, pl. 7.

——— and BAKER, A. C.

1913. Classification of the Aleyrodidae. Pt. 1. U. S. Dept. Agr. Bur. Ent. [Bul.] 27, pt. 1, Tech. Ser., 93 p., 11 fig., 34 pl.

[RILEY, C. V., and HOWARD, L. O.]

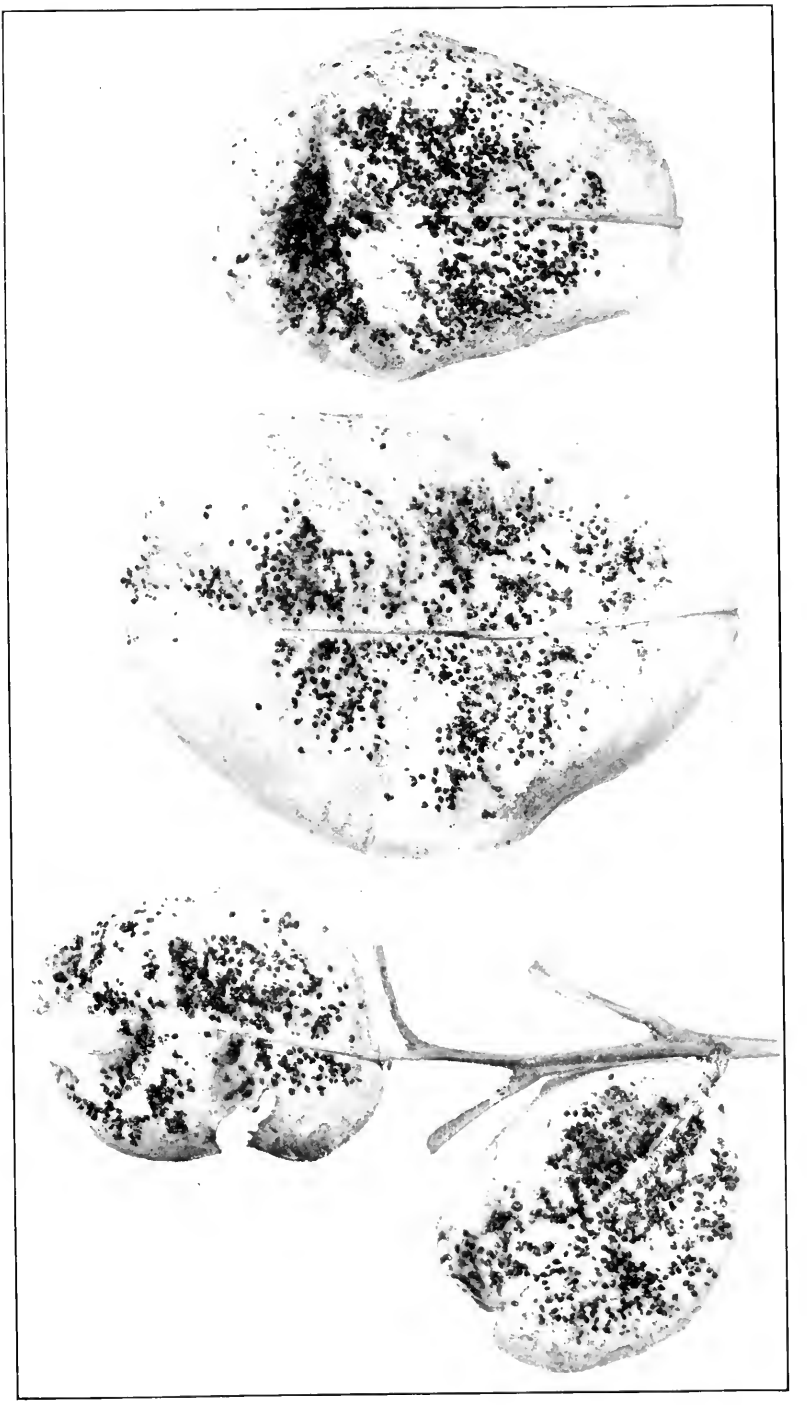
1893. The orange Aleyrodes. *In* Insect Life, v. 5, no. 4, p. 219–226.

WELDON, G. P.

1915. White fly at Marysville. *In* Mo. Bul. State Com. Hort. [Cal.], v. 4, no. 8, p. 386–388.

PLATE LXIV

Aleurocanthus woglumi: Eggs, larvæ, and pupa cases on orange leaves.



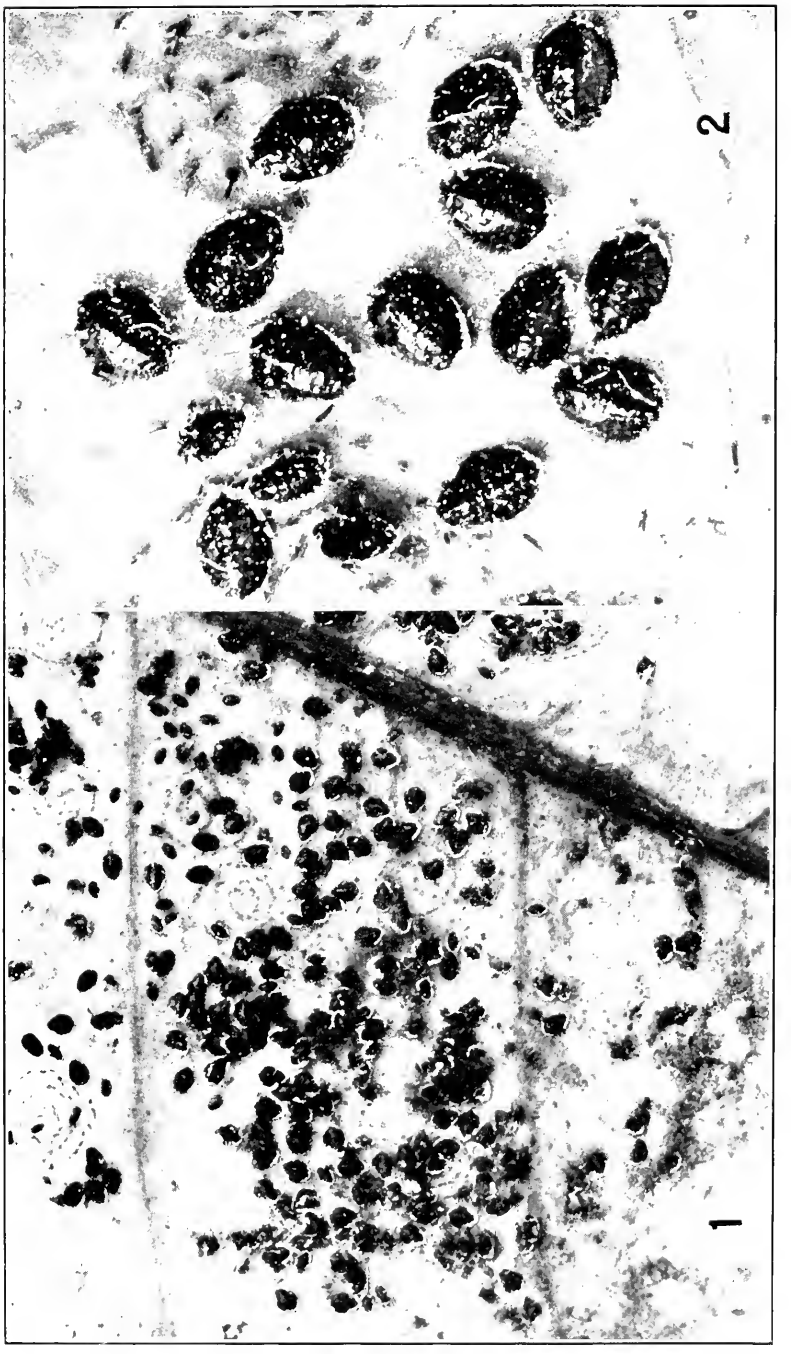

2
1

PLATE LXV

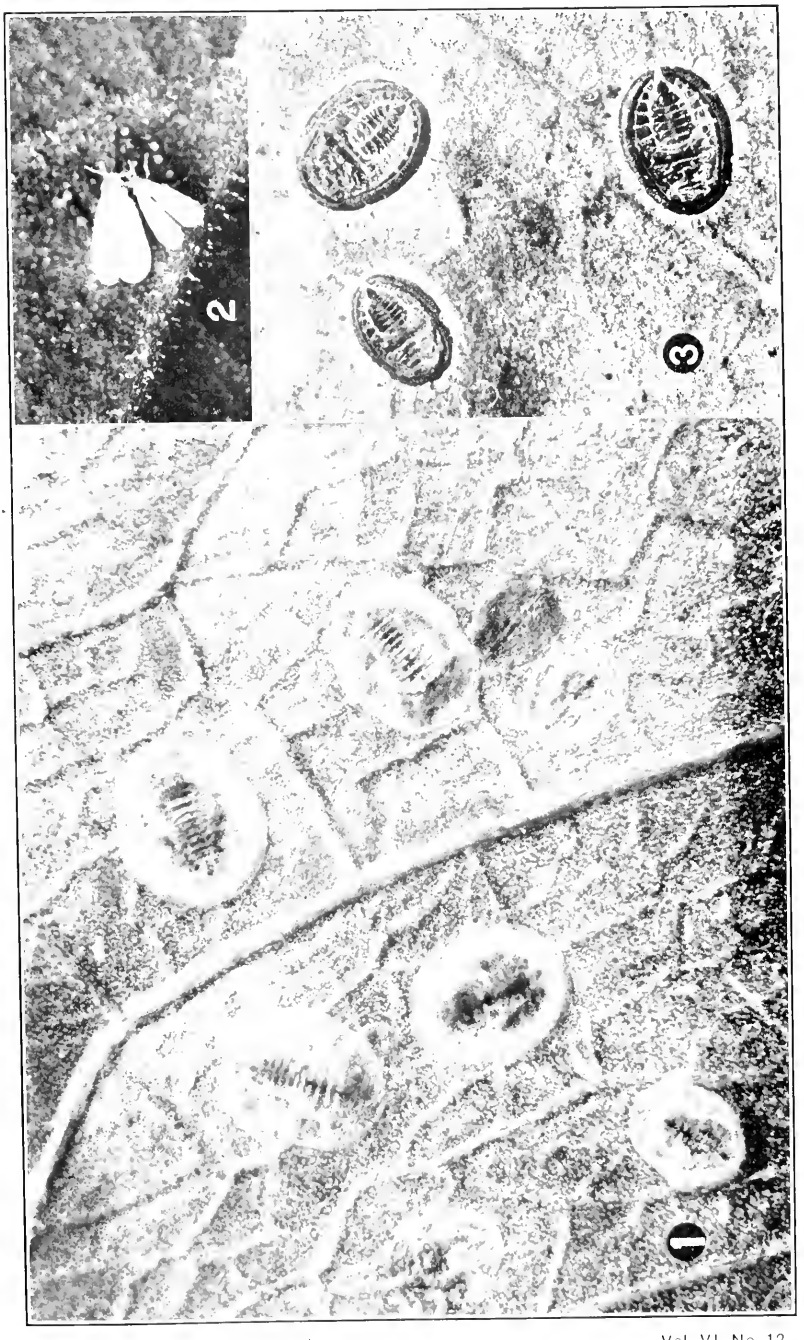
Aleurocanthus woglumi:

Fig. 1.—Colony on an orange leaf.
Fig. 2.—Eggs and pupa cases, greatly enlarged

37769°—16——4

PLATE LXVI

Fig. 1.—*Dialeurodes citri:* Pupæ, much enlarged.
Fig. 2.—Male and female adults of an aleyrodid.
Fig. 3.—*Aleurolobus marlatti,* much enlarged.


2
3
1

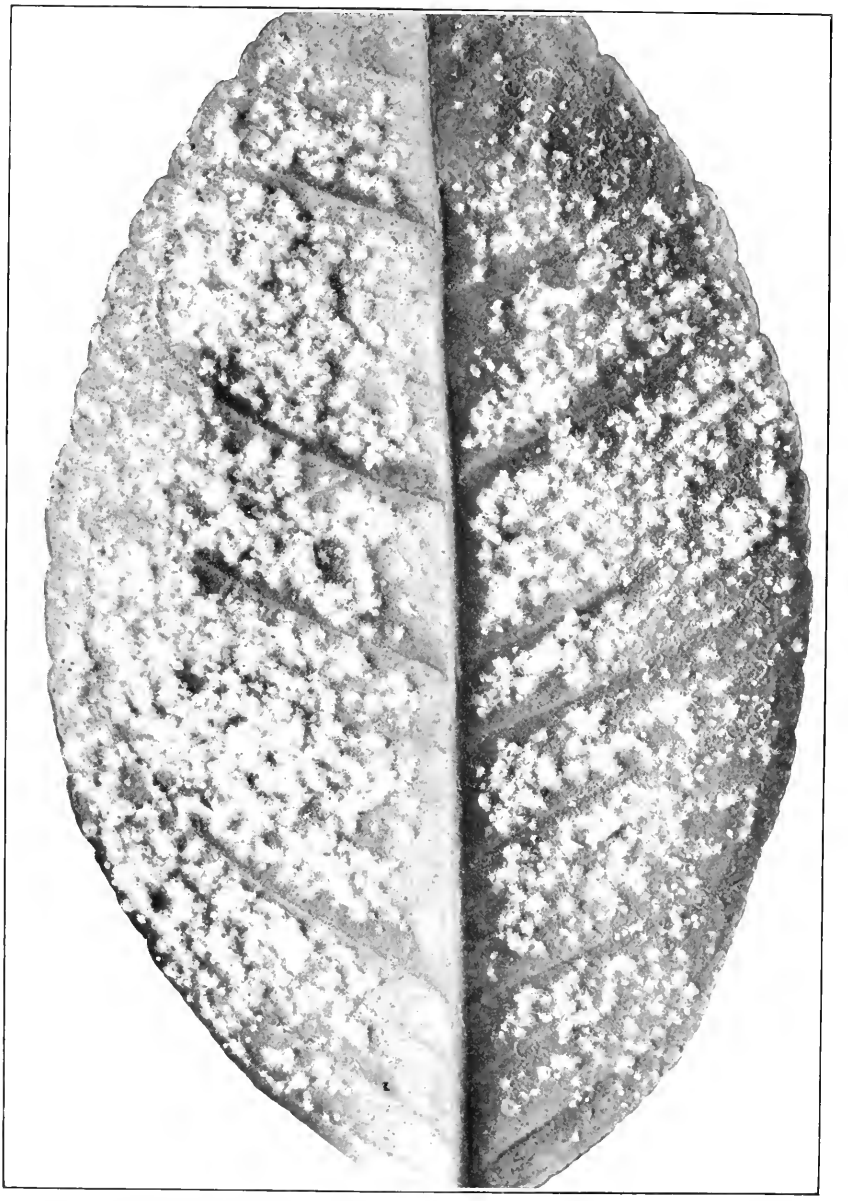
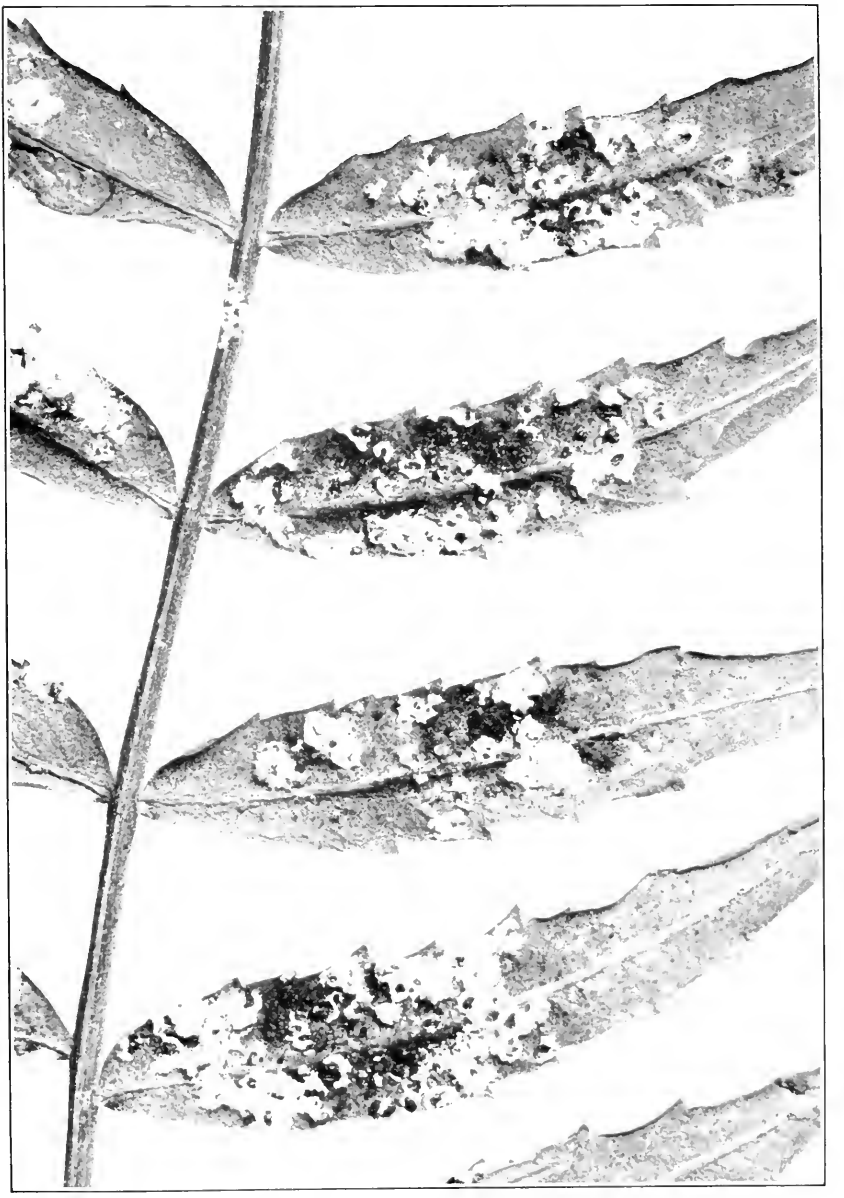


PLATE LXVII

Aleurothrixus howardi: Larvæ and pupa cases on an orange leaf, enlarged.

PLATE LXVIII

Aleurothrixus porteri: Larvæ and pupa cases on *Myrtus* sp., enlarged.



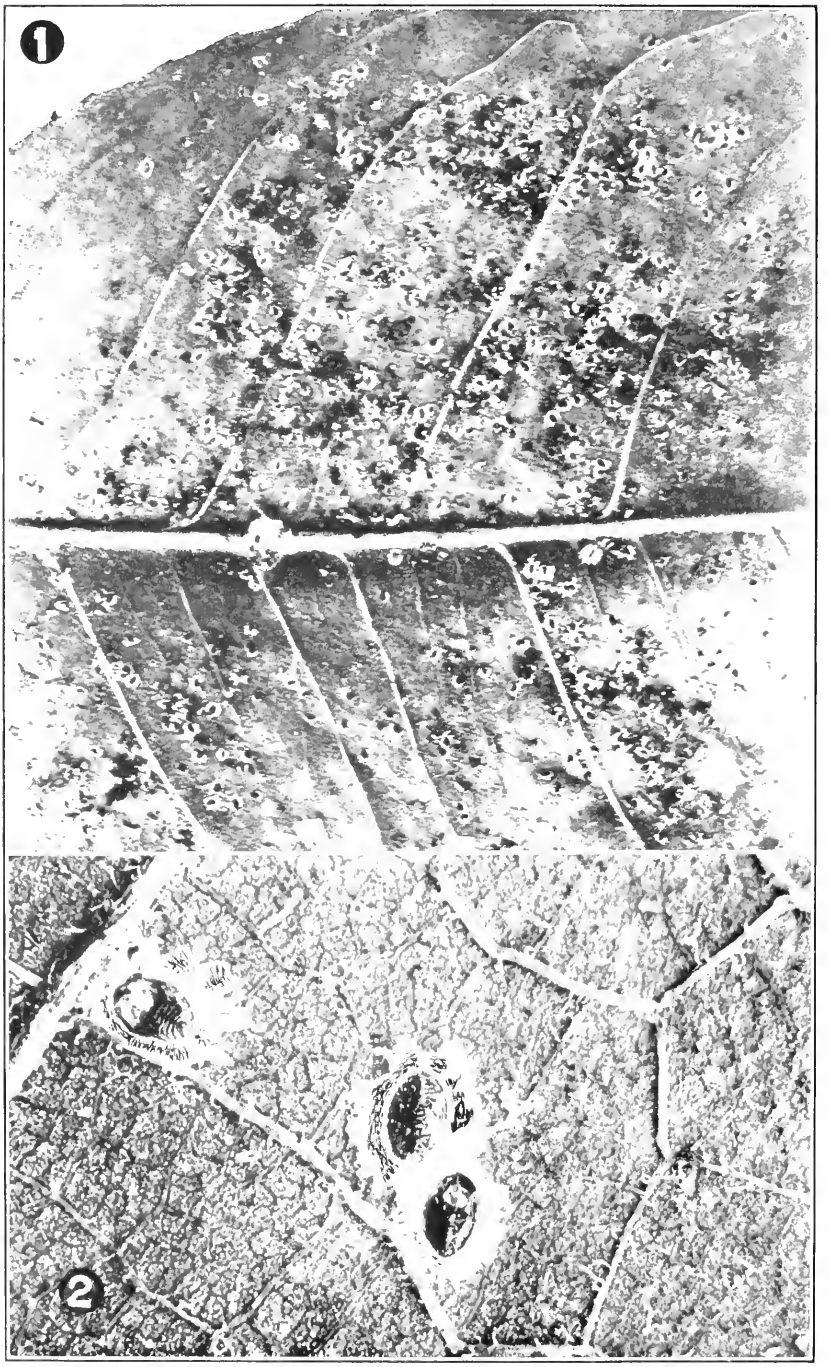

1
2

PLATE LXIX

Fig. 1.—*Tetraleurodes mori*, var *arizonensis:* Larvæ and pupa cases on an orange leaf, enlarged.

Fig. 2.—*Tetraleurodes mori:* Pupa cases on a mulberry leaf, much enlarged.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

25 CENTS PER COPY

SUBSCRIPTION PRICE, $3.00 PER YEAR

▽

Vol. VI JUNE 26, 1916 No. 13

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1916

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

EDITORIAL COMMITTEE

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from Experiment Stations should be addressed to Raymond Pearl, Journal of Agricultural Research, Orono, Maine.

JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., JUNE 26, 1916 NO. 13

RELATIVE WATER REQUIREMENT OF CORN AND THE SORGHUMS

By EDWIN C. MILLER

Assistant Plant Physiologist, Department of Botany, Kansas Agricultural Experiment Station

INTRODUCTION

During the summers of 1914 and 1915 a physiological study of the water relations of corn and the nonsaccharin sorghums was made at the State Branch Experiment Station at Garden City, Kans. In connection with other experiments it was thought advisable to determine the water requirement of several varieties of these plants. The term "water requirement," as used in this paper, means the ratio of the weight of the water absorbed by the plant to the weight of the dry matter produced.

EXPERIMENTAL METHODS

CLIMATIC DATA

The instruments for recording the climatic conditions consisted of a hydrograph, a thermograph, maximum and minimum thermometers placed in a standard shelter 4 feet from the ground, a rain gauge, an evaporation tank, and an anemometer which measured the wind velocity at a height of 2 feet.

A portion of the weather records for the two seasons averaged for five-day periods is shown in Table I. These show that the climatic conditions for the two seasons were in marked contrast. The summer of 1915 was much cooler than that of 1914 and the rainfall for the months of May, June, July, August, and September in 1915 was approximately three times that for the same months in 1914. The evaporation during 5-day periods is shown graphically in figure 1.

The evaporation for each of the growing months with but one exception was much higher in 1914 than in 1915.

CULTURAL METHODS

The plants were grown in large metal cans made from 22-gauge galvanized iron. These cans were 24 inches in height with a diameter of 15 inches, and under the conditions of these experiments contained about

110 kgm. of soil. Forty of these cans were used in 1914 and 60 in 1915. The upper foot of field soil was worked through a sieve with a ¼-inch mesh and then thoroughly tamped in the cans. The soil was in good tilth, and for both seasons the moisture content ranged from 20 to 21 per cent (dry basis). It had a moisture equivalent of 24 and a wilting coefficient of 13, as calculated by the formula of Briggs and Shantz.[1]

The cans were provided with metal lids which were sealed with ordinary binding tape (Pl. LXXII, fig. 4). This was made waterproof by

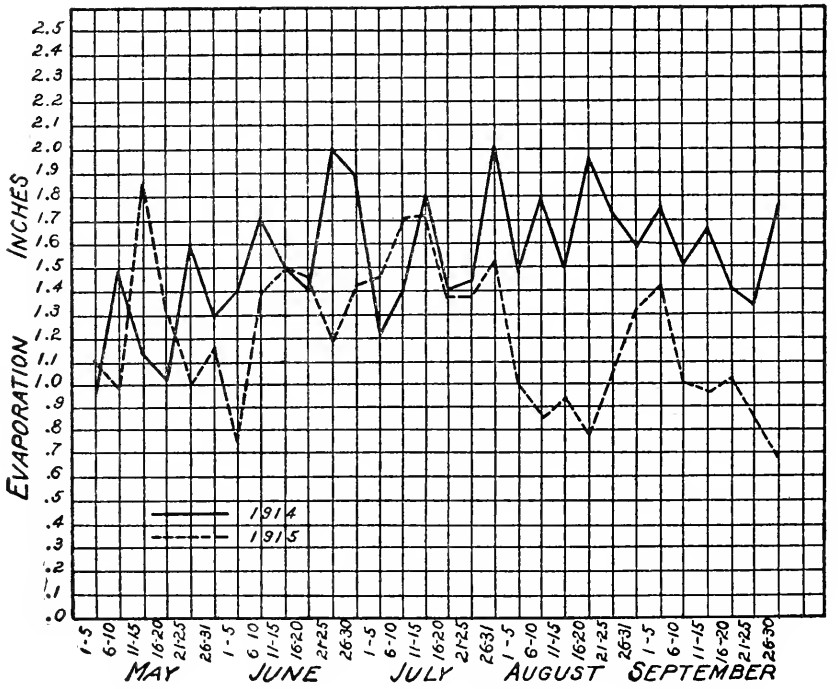


FIG. 1.—Curves of the evaporation at Garden City, Kans., for the growing period of 1915.

giving it a heavy coat of varnish after it was in position. Three 2-inch holes equidistant from one another were made near the periphery of each lid to accomodate the plants. The seeds were planted in the cans and the young plants gradually thinned to the desired number. Three corn plants were grown in each can, both in 1914 and 1915. Six sorghum plants were grown in each can in 1914, but in 1915 the number was reduced to three plants to each can. In order that the plants might be as nearly as possible under the same climatic conditions during the growing season, the seeds of all the plants used were sowed on the same date. These were planted on May 26 in 1914, and on May 22 in 1915.

[1] Briggs, L. J., and Shantz, H. L. The wilting coefficient for different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230, 83 p., 9 fig., 2 pl. 1912.

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for 1914 and 1915*

Period (inclusive).	Air temperature (°F.).					Precipitation.	Evaporation.	Wind velocity per hour.
	Average of—			Maximum.	Minimum.			
	Means.	Maximums.	Minimums.					
1914.								
May:						*Inches.*	*Inches.*	*Miles.*
1 to 5	58	68	47	78	44	1.40	0.953	9.0
6 to 10	65	78	51	92	41	.19	1.484	11.8
11 to 15	53	61	44	72	38	.20	1.135	10.9
16 to 20	62	68	55	79	50	.72	.596	13.6
21 to 25	72	84	59	90	57	.12	1.584	10.2
25 to 31	69	79	57	89	49	1.00	1.294	6.9
June:								
1 to 5	76	87	65	92	62	.19	1.432	13.0
6 to 10	77	89	64	91	51	.21	1.728	15.2
11 to 15	76	88	63	96	59	.61	1.520	9.3
16 to 20	76	89	62	99	58	.39	1.409	6.4
21 to 25	82	94	69	98	64	.....	1.991	9.9
26 to 30	77	94	59	103	51	.04	1.862	7.7
July:								
1 to 5	74	85	62	94	53	.15	1.200	6.1
6 to 10	77	91	60	93	53	.10	1.440	4.7
11 to 15	86	99	69	103	64	.....	1.822	5.7
16 to 20	76	87	62	101	58	.21	1.416	7.7
21 to 25	81	94	65	98	64	.10	1.451	5.7
26 to 31	83	98	66	102	64	Trace.	2.074	5.7
August:								
1 to 5	77	93	65	95	61	.38	1.477	6.1
6 to 10	77	91	62	95	56	Trace.	1.792	8.0
11 to 15	77	91	62	95	58	.19	1.474	7.0
16 to 20	82	99	64	102	62	.06	1.959	8.2
21 to 25	77	91	61	99	50	.01	1.745	7.5
25 to 31	73	87	60	94	54	Trace.	1.563	7.4
September:								
1 to 5	77	94	60	103	55	.....	1.739	7.5
6 to 10	79	96	64	102	59	.01	1.501	8.6
11 to 15	75	89	58	96	48	.03	1.653	11.4
16 to 20	77	90	60	97	56	.....	1.390	7.6
21 to 25	63	80	44	85	37	.11	1.343	6.4
26 to 30	67	86	51	90	47	.....	1.740	11.1
1915.								
May:								
1 to 5	53	65	38	76	31	.79	1.187	10.0
6 to 10	56	69	44	81	32	.....	.985	7.7
11 to 15	71	87	55	94	46	.....	1.857	10.8
16 to 20	46	55	39	68	32	2.38	1.324	12.2
20 to 25	67	78	57	90	44	.07	1.069	8.6
25 to 31	55	65	47	72	39	1.15	1.169	8.1
June:								
1 to 5	65	75	58	81	55	.64	.738	8.7
6 to 10	64	78	52	86	36	.94	1.386	8.6
11 to 15	66	78	53	87	50	.....	1.490	8.0
16 to 20	71	85	61	95	56	.07	1.485	8.8
21 to 25	69	79	58	91	56	.62	1.181	8.5
26 to 30	72	84	59	88	57	.69	1.419	7.1
July:								
1 to 5	66	77	55	83	49	.57	1.451	8.8
6 to 10	76	90	60	96	54	.51	1.732	8.6

TABLE I.—*Summary of the climatic conditions at Garden City, Kans., for 1914 and 1915*—Continued

Period (inclusive).	Air temperature (°F).					Precipitation.	Evaporation.	Wind velocity per hour.
	Average of—			Maximum.	Minimum.			
	Means.	Maximums.	Minimums.					
1915.								
July—Continued.						*Inches.*	*Inches.*	*Miles.*
11 to 15	81	97	67	101	64	0.06	1.743	6.7
16 to 20	72	84	62	96	56	.15	1.407	7.0
21 to 25	74	85	61	91	56	.13	1.397	5.5
25 to 31	75	74	64	90	62	.24	1.528	6.8
August:								
1 to 5	69	83	56	90	51	.90	1.012	5.8
6 to 10	70	80	60	94	56	5.11	.860	4.9
11 to 15	72	83	61	86	59	.10	.927	2.7
16 to 20	61	80	61	84	57	.03	.790	3.2
21 to 25	70	81	60	84	57	.46	1.018	4.4
25 to 31	63	77	50	85	40	.....	1.313	4.7
September:								
1 to 5	68	83	55	87	51	.82	1.424	7.4
6 to 10	69	81	56	91	54	.....	1.029	6.3
11 to 15	71	84	60	97	53	Trace.	.983	7.2
16 to 20	69	82	55	87	39	.20	1.072	5.2
21 to 25	66	76	58	84	50	1.00	.864	18.2
25 to 30	56	67	48	78	44	.25	.665	4.4

The holes in the lids were made water-tight by using a mixture of approximately 16 parts by weight of beeswax to 1 part of Venetian turpentine. Under ordinary conditions the young seedlings of the corn and sorghum can readily penetrate this wax. After the plants had emerged through the wax, it was replaced by a mixture containing a much smaller amount of Venetian turpentine, in order to secure a seal that would remain firm around the plants during the hot summer weather. The lids of the cans were given a heavy coat of white paint and were then covered with a layer of burlap in order to protect them from excessive heat. The water lost by the plants was replaced every 48 hours by the method used by Briggs and Shantz [1] in their extensive work on the water requirement of plants.

It was thought advisable to determine the water requirement based on the dry weight of both the aerial portions and the roots of the plants. The water requirement was obtained in this manner for Pride of Saline corn, Blackhull kafir, Dwarf milo, and Dwarf Blackhull kafir. The method used in the isolation of the root systems of these plants has been previously reported by the writer int his Journal.[2]

[1] Briggs, L. J., and Shantz, H. L. The water requirement of plants. I. Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, 49 p., 2 fig., 11 pl. 1913.

——— ——— The water requirement of plants. II. A review of the literature. U. S. Dept. Agr. Bur. Plant Indus. Bul. 285, 96 p., 5 fig. 1913.

——— ——— The relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1–64, 1 fig., pl. 1–7. 1914.

[2] Miller, E. C. A comparative study of the root systems and leaf areas of corn and the sorghums. *In* Jour. Agr. Research, v. 6, no. 9, p. 311–332. 1916.

SCREENED INCLOSURE

The plants were grown in a screened shelter in order to protect them from the hailstorms and severe winds that are frequent in this region. The inclosure was 20 feet square and had a flat top 10 feet from the ground. It consisted of a framework of 2 by 4 inch studding spaced 3 feet apart and covered on both the top and sides by a wire netting with a ¼-inch mesh. Cheesecloth was placed around the sides of the inclosure to a height of 4½ feet from the ground. This was held in position by poultry netting tacked over the outside (Pl. LXX, fig. 1).

The bottom of the inclosure was provided with a smooth, rigid floor made of matched pine lumber. The cans were placed in three double rows running north and south inside the inclosure, with a space of 2 feet between each row. The height of the floor was such that the upper surface of the cans came to within 1½ feet of the top of the cheesecloth.

The rate of evaporation inside and outside the shelter was determined by two Livingston [1] porous-cup atmometers. These were renewed every three or four weeks. They were connected with burettes which were graduated to 0.1 c. c., and readings were made twice each day. The atmometer outside the inclosure was placed at a distance of 2 feet from the ground in the center of a plot that was planted to corn. The atmometer in the inclosure was placed a few inches above the upper surface of the cans during the early part of the growing season and 2 feet above their tops when the plants had reached 3 feet in height. The monthly evaporation for the two seasons from the porous-cup atmometers, having a coefficient of 0.74 is given in Table II.

TABLE II.—*Monthly evaporation (in cubic centimeters) inside and outside the screened inclosure for 1914 and 1915*

Period.	Outside.	Inside.	Ratio.
1914.			
June 10 to July 10	2,595	1,494	1.7
July 10 to Aug. 10	2,317	1,593	1.4
Aug. 10 to Sept. 10	2,124	1,462	1.4
1915.			
June 10 to July 10	2,028	1,274	1.5
July 10 to Aug. 10	1,627	1,233	1.3
Aug. 10 to Sept. 10	1,589	976	1.6

The rate of evaporation within the inclosure as measured by the porous-cup atmometers, was only approximately two-thirds as high as that in the field. Briggs and Shantz [2] found that plants grown in such a shelter had a water requirement approximately 20 per cent lower than

[1] Livingston, B. E. The Relation of Desert Plants to Soil Moisture and to Evaporation. 78 p., illus., Washington, D. C., 1906. (Carnegie Inst. Washington, Pub. 50.) Literature cited, p. 77–78.
——— Operation of the porous-cup atmometer. *In* Plant World, v. 13, no. 5, p. 111–119. 1910.

[2] Briggs, L. J., and Shantz, H. L., 1913. Op. cit.

plants of the same kind grown in the open. The relative water requirment, however, is probably affected little, if at all, by the shading due to an inclosure of this kind, and it offers the only scientific method for studying the relative transpiration of plants under the severe climatic conditions experienced in this region.

WEIGHING THE CANS

Each can was placed on a small wooden platform, which was provided with a screw eye at either end and mounted on four iron castors. By means of an iron rod, hooked at one end and bent into a handhold at the other, the cans could be moved easily wherever desired (Pl. LXX, fig. 2). The cans were pulled over a track made of pine flooring to a small scale house located 12 feet from the shelter and were weighed every 48 hours on platform scales that were sensitive to 50 gm. (Pl. LXX, fig. 1). In this manner two men could easily weigh the 60 cans in less than 1½ hours.

EXPERIMENTAL DATA

CORN

Four varieties of corn were grown in 1914 and three varieties in 1915. The results for the two years are shown in Tables III and IV.

TABLE III.—*Water requirement of Pride of Saline corn at Garden City, Kans., in 1914 and 1915*

Period of growth.	Pot No.	Number of plants.	Dry matter, including roots.	Dry matter, without root (stem and leaves).	Water transpired.	Water requirement based on—	
						Total dry weight, including roots.	Total dry weight, excluding roots (stem and leaves).
1914.			*Gm.*	*Gm.*	*Kgm.*		
May 26 to Aug. 22.	13	3	164. 3	150. 6	53. 5	325. 8	355. 4
	14	3	169. 8	153. 9	63. 3	373. 1	411. 7
	15	3	147. 0	131. 4	61. 7	420. 0	469. 9
	16	3	180. 1	163. 7	61. 4	341. 0	375. 3
Mean.....	.....	.....	.....	.....	.....	365±15	403±18
1915.							
May 22 to Aug. 25.	17	3	236. 1	205. 6	56. 9	241. 1	276. 0
	18	3	285. 6	252. 5	64. 2	225. 1	254. 6
	19	3	260. 4	234. 4	63. 4	243. 7	270. 7
	20	3	230. 6	202. 4	55. 3	240. 0	273. 4
	21	3	244. 3	211. 2	58. 1	238. 1	275. 5
	22	3	260. 0	228. 3	59. 2	227. 9	259. 6
	23	3	.....	165. 4	46. 6	.....	282. 2
	24	3	.....	178. 6	46. 0	.....	257. 9
	25	3	.....	180. 5	46. 7	.....	259. 0
	26	3	.....	154. 4	40. 6	.....	263. 0
Mean.....	.....	.....	.....	.....	.....	236±3	267±2

TABLE IV.—*Water requirement of Sherrods White Dent, Chinese, and hybrid corn at Garden City, Kans., in 1914 and 1915*

Variety and period of growth.	Pot No.	Number of plants.	Dry matter, excluding roots (stem and leaves).	Water transpired.	Water requirement.
1914.			*Gm.*	*Kgm.*	
Sherrods White Dent, May 26 to Aug. 22	17	3	133.3	54.7	410.8
	18	3	142.4	50.7	356.3
	19	3	143.7	60.8	423.3
Mean					396±16
Chinese, May 26 to Aug. 22	20	3	136.1	58.1	427.1
	21	3	157.3	64.5	410.3
Mean					418±7
Hybrid $F_3 H_{58}$,[a] May 26 to Aug. 22	22	3	120.1	40.2	335.3
	23	3	143.3	50.5	361.8
	24	3	142.6	54.4	381.4
	25	3	155.0	51.9	342.0
Mean					355±8
1915.					
Sherrods White Dent, May 22 to Aug. 18	27	3	145.7	42.8	293.7
	28	3	150.4	43.7	291.0
	29	3	145.2	41.0	282.9
	30	3	120.5	39.8	330.9
Mean					299±8
Hybrid $F_4 H_{58}$,[a] May 22 to Aug. 25	43	3	239.7	54.1	225.9
	44	3	125.6	33.3	265.7
	45	3	137.7	36.3	264.0
	46	3	248.5	58.0	233.4
	47	3	249.3	60.7	243.7
Mean					246±6

[a] This hybrid has the following origin: The female parent was a plant belonging to the F_1 generation of a cross between Sherrods White Dent corn ♀ and white Chinese corn ♂. The male parent was a plant of the variety known as Esperanza (Mexican corn). The cross was made on the breeding grounds of the Department of Botany of the Kansas Experiment Station in 1910.

Four cans of Pride of Saline corn were grown in 1914 and ten in 1915. These plants varied in mature height from 5 to 6 feet, but produced no ears during either season. The plants grew from May 26 to August 22 in 1914, and from May 22 to August 25 in 1915. The water requirement of Pride of Saline corn, based on the total dry matter, including the roots, was found to be 365±15 in 1914 and 236±3 in 1915. The water requirement, based on the total dry matter of the aerial parts of the plants, was 403±18 and 267±2 for the years 1914 and 1915, respectively (Pl. LXXII, fig. 2).

Sherrods White Dent corn was grown in three cans in 1914 and in four cans in 1915. In 1914 the seeds were planted on May 26 and the

plants were harvested on August 22, while in 1915 they were planted on May 22 and harvested on August 18. The water requirement of this variety of corn, based on the total dry matter of the aerial parts, was found to be 396±16 in 1914 and 299±8 in 1915.

In 1914 two cans were planted to white Chinese corn. The growing season of these plants was from May 26 to August 22. The water requirement, based on the dry weight of the aerial parts, was 418±7.

In 1914 four cans were planted to the F_3 generation of a segregate of a hybrid corn developed by the Department of Botany of the Kansas Experiment Station. Five cans of the F_4 generation of this hybrid were grown in 1915. Its water requirement, based on the total dry matter of the aerial parts, was 355±8 and 246±6, for the years 1914 and 1915, respectively.

SORGHUMS

Dwarf milo and Blackhull kafir were the only sorghums grown in 1914. In addition to these two varieties, dwarf black-hulled white kafir, feterita, and sudan grass were grown in 1915. The results for the two seasons are shown in Tables V and VI.

Six cans of Dwarf milo were planted in 1914 and eight cans in 1915. The plants in the former year reached a height of 3 feet, and during the latter year they stood 4½ feet high (Pl. LXXI, fig. 1). The growing season was from May 26 to August 22 in 1914, and from May 22 to September 3 in 1915. The water requirement, based on the total dry matter, including the roots, was found to be 319±5 in the former year and 228±3 in the latter. The water requirement, based on the total dry matter of the aerial parts, was 340±5 and 244±3 for the years 1914 and 1915, respectively. The water requirement, based on the production of grain, was 1,022±100 in 1914 and 508±6 in 1915.

Blackhull kafir was grown in six cans in 1914 and in eight cans in 1915. The seed was planted on May 26 and the plants were harvested on September 3 in 1914, while in 1915 the growing period was from May 22 to September 18. The plants reached a height of 6 feet in each of the growing seasons (Pl. LXXII, fig. 3). The water requirement, based on the total dry matter, including the roots, was 305±6 in 1914 and 204±2 in 1915, while the water requirement, based on the total dry weight of the aerial parts, was 325±7 for the former year and 217±2 for the latter. The water requirement, based on the production of grain, was 1,178±45 in 1914 and 696±19 in 1915.

TABLE V.—*Water requirement of Dwarf milo and Blackhull kafir at Garden City, Kans., in 1914 and 1915*

DWARF MILO

Period of growth.	Pot No.	Number of plants.	Dry matter, including roots.	Dry matter, without roots.	Grain.	Stem and leaves.	Water transpired.	Water requirement based on— Total dry weight, including roots.	Total dry weight, excluding roots.	Grain.	Stem and leaves.
1914.			*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Kgm.*				
May 26 to August 22..	1	6	199. 0	187. 3	71. 8	115. 5	61. 4	308. 9	328. 2	855. 3	532. 2
	2	6	172. 2	161. 5	40. 4	121. 1	55. 8	324. 2	345. 6	1, 381. 9	461. 0
	3	6	186. 8	173. 7	45. 0	128. 9	65. 1	348. 7	374. 6	1, 447. 7	505. 4
	4	6	196. 4	184. 4	79. 3	105. 1	60. 6	308. 7	328. 7	764. 4	576. 8
	5	6	173. 7	161. 7	58. 8	102. 9	51. 6	297. 5	319. 9	879. 0	502. 3
	6	6	169. 2	159. 7	68. 5	91. 2	55. 1	326. 0	345. 3	805. 2	604. 8
Mean..								319±5	340±5	1022±100	530±15
1915.											
May 22 to September 3...	1	3	228. 1	214. 6	103. 1	111. 5	51. 5	225. 7	239. 9	499. 5	461. 8
	2	3	239. 1	226. 4	114. 6	111. 8	50. 5	211. 5	223. 3	437. 2	452. 3
	3	3	245. 4	231. 4	105. 6	125. 8	55. 3	225. 7	239. 3	524. 5	440. 3
	4	3	245. 9	223. 3	102. 0	121. 3	56. 1	228. 2	251. 3	549. 1	462. 6
	5	3	248. 3	233. 3	109. 6	123. 7	55. 6	224. 1	238. 5	507. 7	449. 8
	6	3	231. 6	217. 6	107. 6	110. 0	54. 7	236. 3	251. 5	508. 7	497. 6
	7	3	247. 3	230. 5	114. 7	115. 8	60. 4	244. 4	262. 2	526. 7	521. 8
	8	3	240. 8	225. 8	108. 3	117. 5	55. 3	229. 8	245. 1	511. 0	471. 0
Mean..								228±3	244±3	508±6	469±7

BLACKHULL KAFIR

1914.											
May 26 to September 3...	7	4	234. 4	217. 9	54. 5	163. 4	75. 0	320. 0	344. 2	1, 376. 4	459. 1
	8	5	247. 0	234. 1	66. 7	167. 4	68. 1	276. 0	291. 2	1, 022. 1	407. 3
	9	5	226. 8	212. 6	55. 5	157. 1	67. 7	298. 7	318. 7	1, 220. 9	431. 3
	10	6	233. 3	219. 5	60. 5	159. 0	78. 1	335. 0	356. 0	1, 291. 9	491. 5
	11	4	186. 5	175. 6	52. 0	123. 6	58. 6	314. 5	334. 1	1, 126. 7	474. 6
	12	6	278. 1	257. 3	77. 3	180. 0	79. 8	287. 0	310. 0	1, 032. 4	443. 2
Mean..								305±6	325±7	1, 178±45	451±7
1915.											
May 22 to September 18..	9	3	360. 7	341. 7	126. 7	215. 0	74. 8	207. 5	219. 1	590. 9	348. 2
	10	2	233. 9	219. 3	72. 1	147. 2	49. 2	210. 7	224. 7	683. 6	334. 8
	11	3	324. 7	299. 7	92. 4	207. 3	67. 8	208. 9	226. 3	734. 3	327. 3
	12	3	311. 3	287. 8	81. 2	206. 6	64. 5	207. 3	224. 2	794. 8	312. 3
	13	3	325. 0	310. 3	97. 2	213. 1	67. 9	208. 9	218. 8	698. 5	318. 6
	14	3	363. 8	342. 8	89. 6	253. 2	70. 9	194. 9	206. 8	791. 5	280. 0
	15	3	353. 9	333. 8	114. 3	219. 5	70. 6	199. 5	211. 5	617. 7	307. 6
	16	3	368. 9	354. 2	109. 6	244. 6	72. 0	195. 2	203. 3	657. 3	294. 5
Mean..								204±2	217±2	696±19	315±5

TABLE VI.—*Water requirement of Dwarf Blackhull kafir, feterita, and Sudan grass at Garden City, Kans., in 1915*

Plant and period of growth.	Pot No.	Number of plants.	Dry matter, including roots.	Dry matter, without roots.	Grain.	Stem and leaves.	Water transpired.	Water requirement based on—			
								Total dry weight, including roots.	Total dry weight, excluding roots.	Grain.	Stem and leaves.
1915.			*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Kgm.*				
Kafir, Dwarf Blackhull, May 22 to September 11.....	31	3	265.7	249.7	107.0	142.7	56.4	212.3	225.9	527.3	395.4
	32	3	235.4	221.8	88.4	133.4	48.3	205.3	217.9	546.8	362.3
	33	3	273.2	257.8	119.9	137.9	56.7	207.3	220.2	473.4	411.6
	34	2	179.2	168.8	71.7	97.1	37.5	209.7	222.6	524.1	387.0
	35	3	247.1	230.2	95.1	135.1	50.0	202.3	217.2	525.8	370.1
Mean..	....	....	.....	.....	.....	.....	.....	207±2	221±2	519±8	385±6
Feterita, May 22 to September 6...	36	3	.....	175.6	59.6	116.0	42.6	.....	242.7	715.2	367.5
	37	3	.....	204.7	66.0	138.7	49.4	.....	242.1	748.4	365.1
	38	3	.....	158.8	55.7	103.1	41.8	.....	263.3	750.8	405.6
	39	2	.....	143.1	42.0	101.1	35.5	.....	248.2	845.7	351.3
	40	3	.....	182.6	52.4	130.2	45.3	.....	248.3	865.2	348.2
Mean..	....	....	.....	.....	.....	.....	.....	.....	249±2	785±24	367±6
Sudan grass, May 22 to September 14.....	41	5	.....	186.4	33.2	153.2	52.5	.....	281.6	1,581.3	342.6
	42	5	.....	173.6	28.4	145.2	50.7	.....	292.5	1,788.3	349.7
	43	5	.....	176.4	42.5	133.9	60.5	.....	343.3	1,425.2	452.3
Mean..	....	....	.....	.....	.....	.....	.....	.....	306±15	1,598±76	381±28

Dwarf Blackhull kafir was grown only in 1915. The growing season for these plants was from May 22 to September 11. The water requirement, based on the total dry matter, including the roots, was 207±2, and based on the total dry weight of the aerial portions, was 221±2. The water requirement, based on the production of grain, was 519±8 (Pl. LXXI, fig. 2).

Feterita was grown in five cans in 1915. The seed was planted on May 22 and the plants were harvested on September 6. The water requirement, based on the total dry matter of the aerial parts, was 249±2, while the water requirement, based on the seed production was 785±24 (Pl. LXXI, fig. 3).

Three cans were planted to Sudan grass in 1915. These plants reached a height of 6 feet during the growing period from May 22 to September 14 (Pl. LXXII, fig. 1). The water requirement, based on the dry weight of the aerial parts, was 306±15 and, based on the production of grain, was 1598±76.

SUMMARY

The water requirement was determined for four varieties of corn and two varieties of sorghum in 1914 and for three varieties of corn and five varieties of sorghum in 1915.

The plants were grown in large sealed galvanized-iron cans which contained approximately 110 kgm. of soil. The soil had a wilting coefficient of 13, and under the conditions of the experiment it had a moisture content of 20 to 21 per cent (dry basis). This moisture content was kept approximately constant by replacing every 48 hours the water that had been lost by transpiration.

Three plants of corn were grown in each can during both seasons. Six sorghum plants were grown to each can in 1914, but in 1915 the number of plants was reduced to three plants to a can.

The plants were grown in a screened inclosure in order to protect them from the hailstorms and severe winds that are prevalent in western Kansas. The rate of evaporation in such a shelter was found to be only two-thirds as high as under field conditions.

The season of 1915 was cooler and more humid, and the rate of evaporation much lower than in 1914. As a consequence the water requirement of the former year was only about 66 per cent of that of the latter year. A summary of the water requirement for the two seasons is given in Table VII.

TABLE VII.—*Summary of the water requirement of the varieties of corn and sorghum grown at Garden City, Kans., in 1914 and 1915*

Plant and period of growth.	Water requirement based on—			
	Dry matter, including roots.	Dry matter, excluding roots.	Grain.	Stem and leaves.
1914.				
CORN:				
Pride of Saline, May 26 to August 22	365±15	403±18	.....	403±18
Sherrods White Dent, May 26 to August 22	.....	396±16	.....	396±16
Hybrid F_3 H_{58}, May 26 to August 22	.....	355± 8	.....	355± 8
Chinese, May 26 to August 22	.....	418± 7	.....	418± 7
KAFIR:				
Blackhull, May 26 to September 3	305± 6	325± 7	1,178± 45	451± 7
MILO:				
Dwarf, May 26 to August 22	319± 5	340± 5	1,022±100	530±15
1915.				
CORN:				
Pride of Saline, May 22 to August 25	236± 3	267± 2	.....	267± 2
Sherrods White Dent, May 22 to August 18	.....	299± 8	.....	299± 8
Hybrid F_4 H_{58}, May 22 to August 25	.....	246± 6	.....	246± 6

TABLE VII.—*Summary of the water requirement of the varieties of corn and sorghum grown at Garden City, Kans., in 1914 and 1915*—Continued

Plant and period of growth.	Water requirement based on—			
	Dry matter, including roots.	Dry matter, excluding roots.	Grain.	Stem and leaves.
1915.				
KAFIR:				
Blackhull, May 22 to September 18.	204± 2	217± 2	696± 19	315± 5
Dwarf Blackhull, May 22 to September 11	207± 2	221± 2	519± 8	385± 6
MILO:				
Dwarf, May 22 to September 3	228± 3	244± 3	508± 6	469± 7
FETERITA:				
May 22 to September 6	.....	249± 2	785± 24	367± 6
SUDAN GRASS: May 22 to September 14	.....	306±15	1,598± 76	381±28

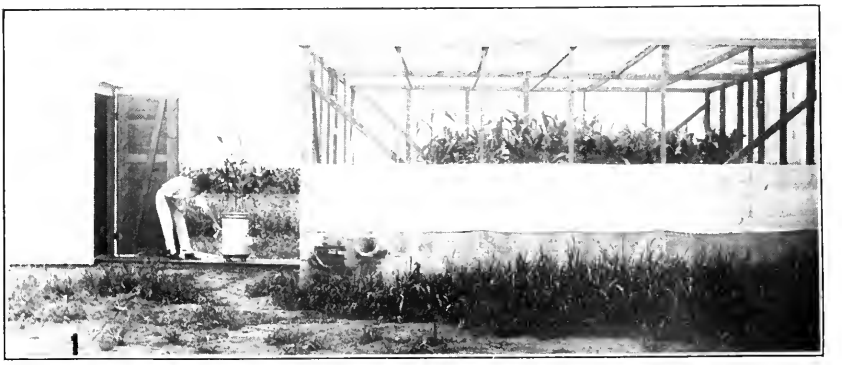
Using the water requirement of Blackhull kafir as 1, the water requirement of the plants grown in 1914 would be as follows: Dwarf milo 1.04, hybrid corn 1.09, Sherrods White Dent corn 1.22, and Pride of Saline corn 1.24. In 1915, if the water requirement of Blackhull kafir be considered as 1, the water requirement of Dwarf Blackhull kafir would be 1.02; Dwarf milo, 1.12; feterita, 1.14; hybrid corn, 1.17; Pride of Saline corn, 1.23; Sherrods White Dent corn, 1.37; and Sudan grass, 1.41.

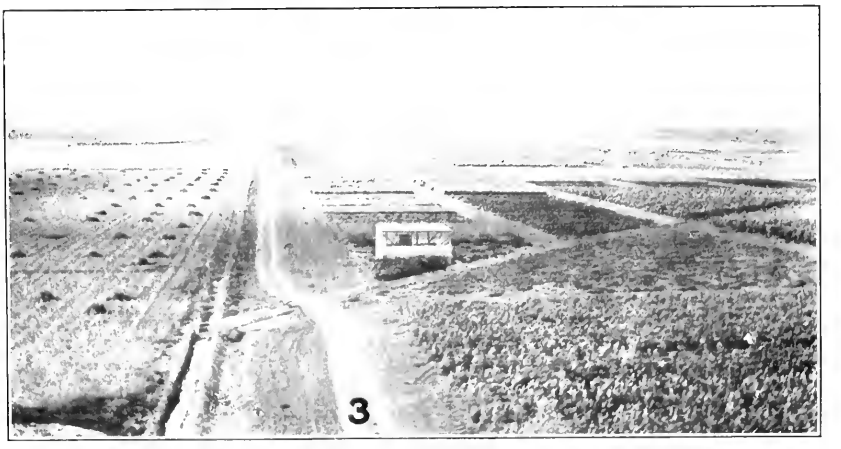
PLATE LXX

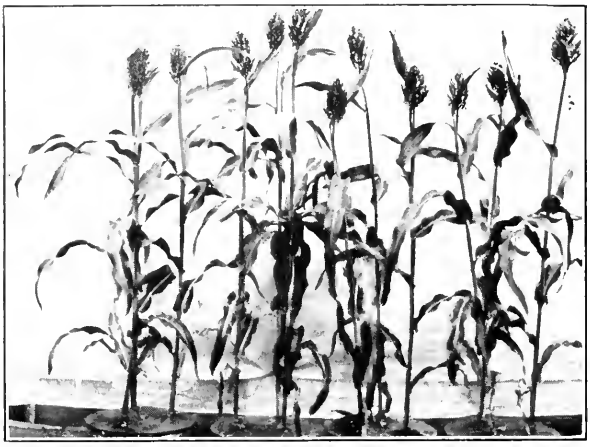
Fig. 1.—General view of the screened inclosure and the scale house.

Fig. 2.—Method of moving the cans.

Fig. 3.—General view of the plant shelter and the surrounding country at Garden City, Kans.







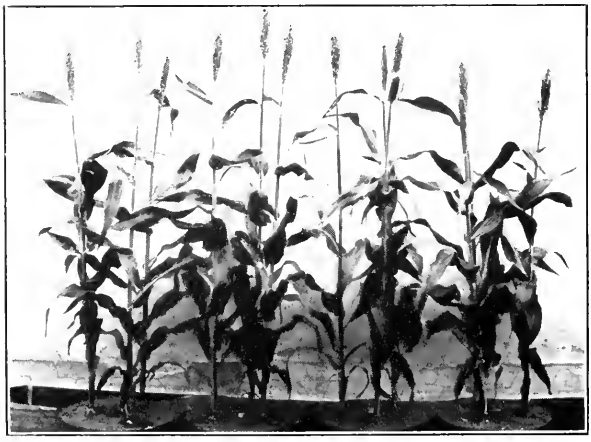


PLATE LXXI

Fig. 1.—Dwarf milo, grown May 22 to September 3, 1915. Water requirement based on total dry matter, including roots, 228±3. Based on dry matter, excluding roots, 244±3. Average of 8 cans.

Fig. 2.—Dwarf Blackhull kafir, grown May 22 to September 11, 1915. Water requirement based on total dry matter, including roots, 207±2. Based on total dry matter, excluding roots, 221±2. Average of 5 cans.

Fig. 3.—Feterita, grown May 22 to September 6, 1915. Water requirement based on total dry matter, excluding roots, 249±2. Average of 5 cans.

PLATE LXXII

Fig. 1.—Sudan grass, grown May 22 to September 14, 1915. Water requirement based on total dry matter, excluding roots, 306±15. Average of 3 cans.

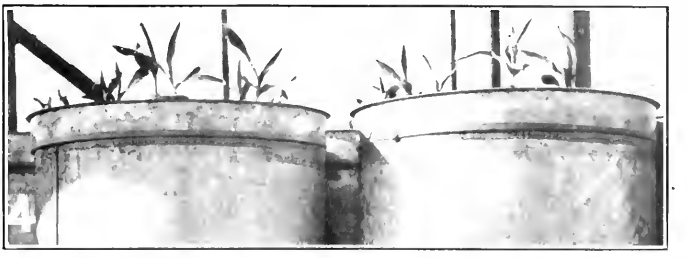
Fig. 2.—Pride of Saline corn, grown May 22 to August 25, 1915. Water requirement based on total dry matter, including roots, 236±3. Based on total dry matter, excluding roots, 267±2. Average of 10 cans.

Fig. 3.—Blackhull kafir, grown May 22 to September 18, 1915. Water requirement based on total dry matter, including roots, 204±2. Based on total dry matter, excluding roots, 217±2. Average of 8 cans.

Fig. 4.—Method of sealing the lids with tape and the wax seal around the plants.


2


3


4

AVAILABILITY OF MINERAL PHOSPHATES FOR PLANT NUTRITION [1]

By W. L. BURLISON,

Associate Professor, Crop Production, Agricultural College, and Associate Chief, Crop Production, Illinois Agricultural Experiment Station [2]

INTRODUCTION

Phosphorus is the key to permanent systems of agriculture for a large portion of the common soils of the corn belt. These soils contain, as an average, 5,000 pounds of nitrogen, 1,200 pounds of phosphorus, and 35,000 pounds of potassium for the surface soil to the depth of 6⅔ inches. If the land were producing corn at the rate of 100 bushels per acre, the nitrogen would be sufficient for 50 crops, the phosphorus for 70 crops, and the potassium for about 1,842 crops. The nitrogen supply can be maintained by the growth and judicious management of leguminous crops. Potassium is present in quantities adequate for many years. With phosphorus the problem is different. This element can not be gathered from the soil air by legumes; nor is it one of unlimited supply. When once removed, phosphorus must be returned to the land in crop residues, in farm manures, or in commercial fertilizers which contain phosphorus.

Since the introduction of commercial fertilizers, more or less discussion has been carried on concerning the value of insoluble mineral phosphates as a source of phosphorus for the nutrition of plants. In Europe (28, p. 329)[3] the highest authorities on agricultural problems have discouraged the use of insoluble phosphates, while in America scientists and practical men have disagreed. Investigations which have been conducted on the use of insoluble minerals are by no means conclusive. Therefore it is the purpose of the work reported in the following pages to throw more light on this question, which is of so great economic importance and scientific significance. The subject matter will be presented according to the following divisions:

I. Review of literature regarding the availability of phosphate minerals.

II. The availability of phosphorus in Tennessee brown rock phosphate for wheat (*Triticum vulgare*), oats (*Avena sativa*), rye (*Secale cereale*), barley (*Hordeum sativum hexastichon*), cowpeas (*Vigna catjang*), soybeans (*Glycine hispida*), timothy (*Phleum pratense*), red clover (*Trifolium pratense*), and alfalfa (*Medicago sativa*).

---

[1] This paper is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agronomy in the Graduate School of the University of Illinois, 1915.

[2] The author wishes to express his appreciation for the suggestions and encouragement tendered by Dr. C. G. Hopkins and Dr. A. L. Whiting, of the Illinois Experiment Station.

[3] Reference is made by number to "Literature cited," p. 513-514.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. VI, No. 13
June 26, 1916
Ill.—3

ef
37770°—16——2

III. A comparative study of the productive powers of six mineral phosphates for farm crops.

IV. The influence of fermenting dextrose and crop residues on the availability of phosphorus in finely ground rock phosphate.

V. The influence of the size of particles on the availability of phosphorus in mineral phosphates.

REVIEW OF LITERATURE

The availability of mineral phosphates for plant nutrition has been under investigation at various institutions for more than half a century. Among the earlier scientists who attempted to determine the availability of the phosphorus in mineral phosphates was Dyer (4), who found that undissolved phosphate produced better returns than dissolved phosphate for swedes and oats. Frear (5) studied the comparative value of various phosphorus carriers for farm crops. Finely ground bone and reverted phosphate produced the largest number of mature stalks of corn, and finely ground bone, the highest yield of ears. Superphosphate and certain mineral or raw phosphates were put in field trials by Johnson (9), and for corn, dissolved bone black was superior to others tested. Bishop (1) grew soybeans in pot cultures and concluded that concentrated phosphate and acid phosphate were more desirable than Florida soft rock and iron and aluminum phosphate. Equivalent amounts of different carriers of phosphate were employed by Hess (7) in a 4-year rotation of corn, oats, wheat, and grass. Finely ground bone gave the highest yields of wheat, with raw rock second. Ground bone was most effective for corn, while for oats insoluble ground bone seemed to be satisfactory. South Carolina rock was very useful for clover. Jordan (10) conducted two experiments at the Maine Station with different forms of phosphate. In the first experiment the minerals were applied in equal quantities. For the first two years the acid phosphate gave the highest returns, but later bone meal took the lead. Raw rock was only about half as productive as the other two. In the second trial equal money values of phosphates were applied; and the author points out that, with but one exception, the raw rock gave larger returns than acid phosphate. The work of Jordan, previously mentioned, was continued by Merrill (15), who used pure sand cultures in the greenhouse. Two facts are clear from Merrill's work. First, plants differ widely in their power to assimilate phosphorus from different phosphates. Second, turnips and rutabagas gave almost as good results with raw rock phosphate as with acid phosphate. Later, at the New York Station, Jordan (11) continued the work which he had begun at the Maine Station. His results are in accord with the work previously reported by himself and Merrill.

In 1890 Goessman (2) outlined what has since become a most extensive investigation, concerning the availability of phosphate minerals. In reporting on this work Brooks says (3, p. 104) that—

> It is possible to produce profitable crops of most kinds by liberal use of natural phosphates, and in a long series of years there might be a considerable money saving in depending, at least in part, upon these rather than upon the higher-priced dissolved phosphates.

Results from a second series of experiments begun in Massachusetts in 1897, along the same line as that outlined by Goessman, indicate that phosphatic slag was "exceedingly available for crops, but the Florida soft phosphate was very inferior. For certain crops, South Carolina rock gave surprisingly good returns * * *."

Prianishnikov (20) states that lupins and peas have a very marked ability to obtain phosphorus from natural phosphate, while wheat and oats must be assisted by the solvent powers of the soil or they can not produce normal crops. Schloessing (22) concludes from his experiments that it is not necessary that phosphate should be in a state of solution, since the roots of plants are able to dissolve the phosphorus compounds without the intervention of water.

Patterson (18) reports results, based on a study of various phosphates, which indicate that reverted phosphate gave the highest average yield for corn, wheat, and hay. South Carolina rock phosphate produced slightly better yields than bone black, and Florida soft rock phosphate was quite available for wheat. Wheeler and Adams (30, 31, 32) found raw phosphate profitable for peas, oats, crimson clover, and Japanese millet when used on unlimed land; but for flat turnips, beets, and cabbage it gave poor yields. They are of the opinion that rock phosphate is likely to be most useful when applied to moist soils rich in organic matter, where legumes, corn, and "possibly wheat and oats are to be grown."

Thorne (24, 25), of Ohio, in 1897 inaugurated a very extensive study of the comparative value of raw rock phosphate and acid phosphate used in conjunction with manure. Where, in computing the yields of corn, wheat, and clover, he took the average of all the unfertilized plots as a basis for comparison, he reports (24, p. 18)—

> By this method of calculation the average increase on Plots 2 [floats plus yard manure] and 3 [floats plus stall manure] combined is found to be practically the same as that on Plots 5 [acid phosphate plus yard manure] and 6 [acid phosphate plus stall manure] combined, but when the larger cost of the acid phosphate is deducted the net gain is a little greater on Plots 2 and 3 [with raw phosphate].

By another method of computing the increase he obtains results less favorable to raw phosphate.

Truog (27) has demonstrated rather clearly that farm crops are variable in their ability to secure phosphorus from different sources. Nine of the ten crops tested by him made a better growth on aluminum phos-

phate than on calcium phosphate, and "six made better growth on iron phosphate than on calcium phosphate."

Under the direction of Hopkins (8), the Illinois Experiment Station is conducting probably the most extensive investigation of any in the world on the use of rock phosphate. Some of the most interesting results were obtained from a field near Galesburg, Knox County, Ill., on brown silt loam prairie soil.

> Phosphorus applied in fine-ground natural rock phosphate in part as top dressing, and with no adequate provision for decaying organic matter, paid only 47 per cent on the investment as an average of the first three years. But it should be kept in mind that the word *investment* is here used in its proper sense, for the phosphorus that was removed in the increase produced was less than 2 per cent of the amount applied, and that removed in the total crops less than one-third. During the last six years, however, the phosphorus has paid 130 per cent on the investment, even though two-thirds of the application remains to positively enrich the soil (8, p. 15).

Newman (16) investigated the use of floats with and without cottonseed meal. He found a marked increase in availability where organic matter was used in conjunction with the mineral phosphate. Later experiments by Newman and Clayton (17) confirmed the above results. Lupton (13) continued the work of Newman, but used acid phosphate as a check on the raw rock phosphate, both with and without organic matter. His results are also in accord with Newman's earlier experiments. Where floats were mixed with cottonseed meal and allowed to ferment, the data seemed to show that the fermentation of the material had very little, if any, influence on the availability of the phosphate. Pfeiffer and Thurman (19) found no beneficial results from composting raw rock phosphate with decaying organic matter. In Canada (23) fermenting manures were found to have only slightly solvent action on composted rock phosphate.

Hartwell and Pember (6) mixed fresh cow manure and floats and allowed them to ferment. They feel that there was practically no increase in the availability of phosphorus in the floats. McDowell (14) also found no increase in the availability of phosphate in finely ground rock phosphate by composting the mineral with cow and horse manure. Tottingham and Hoffmann (26), following the same line of investigation as that which McDowell observed, actually found a decrease in water-soluble phosphorus, but the results were similar with acid phosphate.

Krober (12) was unable to find any increase in availability of mineral phosphates by composting with sawdust and allowing fermentation to proceed. Truog (27) believes that fermented manure has a slightly solvent action on crude phosphate. He also points out that a uniform distribution of the phosphate in the soil will give much better results than that poorly distributed.

Krober (12) shows that the acid-forming bacteria and yeasts are of great value in rendering some of the phosphorus in insoluble phosphate

available. He makes the statement that carbon dioxid was more active than other acids in this respect.

The degree of fineness plays an important part in the availability of the crude phosphates. Jordan (11) proves this quite conclusively. He procured better results from the phosphates which were ground to an impalpable powder. Analysis of the plants showed an increase in the proportion of dry matter to phosphorus as the size of the particles decreased. Voelcker (29) in some of the earliest work says that the efficiency of insoluble calcium phosphate depends upon the minuteness of division; the finer the particles the more energetic will be its action.

EXPERIMENTAL WORK

MEDIUM FOR PLANT GROWTH

Pure white sand was used throughout these experiments as a medium for plant growth. For most of the work this material was leached with a dilute solution of hydrochloric acid for three days to insure the removal of plant food. The sand was then washed with distilled water until there was no trace of acid in the drainage solution. Next it was placed on clean paper until dry, when it was sifted, in order that foreign particles might be removed. Samples were collected for a phosphorus determination from each lot of sand washed, but in no case during the progress of the study was the slightest trace of phosphorus detected.

POTS

Two sizes of pots were used in this investigation. When it was necessary to grow the crop to maturity, the small glass battery jars, approximately 6 inches in diameter and 8 inches in height, proved very satisfactory; but when a grain crop was desired, the 4-gallon stone pots were more suitable. All jars were supplied with adequate drainage.

For the cultures grown in the winter the pots were covered with a coat of black paint, but for the summer series a white coat was placed over the black. The black paint prevented the growth of algæ and the white had a tendency to keep the temperature from becoming excessive within the jars. This precaution was clearly justified, for upon several occasions there was a difference of 5° to 10° in temperature between the black and white pots.

KINDS OF CROPS GROWN

Wheat, oats, rye, barley, timothy, cowpeas, soybeans, clover, and alfalfa—nine common crops that are cultivated on Illinois farms—were grown under various treatments for this investigation. High-grade seed from the previous season's crop was selected for planting, and in all cases the grains were treated with a solution of formalin to prevent smut.

In planting the seed special care was exercised in order to obtain a perfect stand, and in only a few instances was there a failure to get the proper number of plants for each pot. It seems in keeping with accurate methods of research to plant more seeds per pot than would be required for a perfect stand if they all germinated. It is safer to remove the extra plants than to transplant or reseed, and the plants are more likely to be uniform if it is possible to make some choice in thinning them down. An exact record was kept of the number of seeds planted, and all those which failed to germinate were dug out.

For inoculating the legumes, nodules from the same crop as the plant to be infected were crushed and placed in 1 liter of distilled water, and 10 c. c. of this solution were applied to the zone nearest the seed. If the nodules were not available, 300 gm. of soil from a field where the respective legumes had been grown were well shaken with 500 c. c. of water, filtered, and 10 c. c. of this solution were applied in the same manner as indicated above.

PLANT FOODS

The first application of plant food was made when the crops were planted, the others at intervals of two weeks. The plant foods were made up in the following manner:

Nitrogen: Dissolved 80 gm. of ammonium nitrate, 50 gm. of potassium sulphate, and 20 gm. of magnesium sulphate each in 2,500 c. c. of distilled water, and 0.1 gm. of ferric chlorid in 250 c. c. of distilled water. A standard application of these plant foods was 10 c. c. of each of the first three and 1 c. c. of the last diluted as desired. In no case was the solution applied in a concentrated form.

MOISTURE SUPPLY

Throughout the first period of these experiments, the water content of the sand was maintained at 14 per cent by weighing the jar each week. This phase of the details became so burdensome that it was omitted. The method was not accurate, at least during the latter period of growth, because of the irregularity in plant development due to different treatments. Some pots gave off more than 10 times the quantities transpired from others. Satisfactory results were obtained by watering the pots when they needed a supply of moisture and no difficulty was experienced in determining the point where the water content of the sand was below normal.

Whenever weather conditions would permit, the pot cultures were placed on trucks and removed to the cage out of doors.

TIME OF HARVESTING AND HANDLING THE CROP

The time of harvest was governed largely by the condition of the experiment. However, in most instances the same factors which control the time of harvest in general farm practice held true here. The grain

crops developed to full maturity, while the clover and alfalfa were cut for hay. Cowpeas and soybeans grown during the winter months were cut for hay, but those planted in the spring produced a seed crop.

Complete data on time of blooming, time of heading, number of plants, number of stems, and height of plants were collected for a comparison which might be of value in interpreting results, although such records will be omitted from this paper. The total weight of grain and straw, together with photographs, will suffice for drawing conclusions.

After harvesting the pot cultures, the materials were suspended in cheesecloth bags from the roof of the greenhouse for a period of two weeks. This was sufficient time for the product to come to a constant air-dried condition. Usually two weighings at an interval of two days were made as a check to insure accurate results.

ANALYSIS

The plants were first cut fine and then ground in a steel mill until the particles would pass a sieve of 80 meshes to the inch. Next, the materials were thoroughly mixed and samples taken for analytical purposes.

The method for the determination of phosphorus was essentially the Pemberton outline, with slight modifications.

Two gm. of the sample [1] were weighed out and moistened with calcium acetate. The sample was then dried in an electric oven and afterwards transferred to a muffle and there remained until the product was burned to a white ash. The ash was taken up with 5 c. c. of nitric acid and heated on a water bath for several minutes. It was necessary to filter to remove any silica present. From this point the regular procedure followed in the volumetric method was observed.

The mineral phosphates used in this investigation represent six types from different sections of the United States and Canada. The total phosphorus and the phosphorus soluble in citric acid are reported in Table I.

TABLE I.—*Total phosphorus and citric-acid-soluble phosphorus in various kinds of rock phosphate* [2]

Kinds of phosphate.	Phosphorus.	
	Total.	Soluble in citric acid.
Tennessee brown rock phosphate	12. 75	9. 92
Tennessee blue rock phosphate	13. 40	10. 29
Utah rock phosphate	13. 81	8. 66
South Carolina land rock phosphate	13. 75	6. 89
Florida soft rock phosphate	13. 98	10. 55
Canadian apatite	12. 75	5. 57

[1] Two gm. was satisfactory for straw and hay, but for the grain ¼ gm. was sufficient.

[2] Four gm. of each of the mineral phosphates were placed in a 1-liter flask and then 1,000 c. c. of a 0.2 per cent solution of citric acid was poured on the ground rock, where it remained for 48 hours with occasional shaking. Then some of the solution was filtered and 100 c. c. of the filtrate taken for analysis.

AVAILABILITY OF THE PHOSPHORUS IN TENNESSEE BROWN ROCK PHOSPHATE

This series comprises a study of the ability of different crops to secure phosphorus for growth from Tennessee brown rock phosphate without the aid of decaying organic matter. The literature indicates rather clearly that crops differ widely in this respect, but there is but very little direct information from trials conducted under controlled conditions where sand was used as a substitute for soil. The suggestion has been made, also, that there is slight increase in the yield with large application of phosphate. The object of the series reported in Tables II to VI is to present new information on these two important points.

The pots used were the large, glazed 4-gallon jars into which could be placed 22,000 gm. of sand (Pl. LXXIII, LXXIV, LXXV). In this case the sand was not leached with dilute acid, but was washed for several days with distilled water. The rock phosphate was ground sufficiently fine to pass through a sieve of 100 meshes to the inch. On March 20, 1914, the pots were seeded; and after the plants had made satisfactory growth, they were thinned to 15 to each jar.

TABLE II.—*Dry matter and phosphorus content of plant products from wheat and oats*

Pot No.	Phosphate added.	Grain.	Straw.	Phosphorus.					
				Grain.	Straw.	Grain.	Straw.	Total in grain and straw.	Percentage removed.
	Gm.	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Mgm.*	*Mgm.*	*Mgm.*	
SERIES 1A; SPRING WHEAT HARVESTED ON JUNE 29, 1914[a]									
1....	0	0	6.0	.....	.....	.....	.....	.....	.....
2....	0	0	6.5	.....	.....	.....	.....	.....	.....
3....	11	1.0	16.0	.....	.....	.....	.....	.....	.....
4....	11	1.4	19.1	0.260	0.038	3.64	7.26	10.90	0.78
5....	22	4.1	21.9	.....	.....	.....	.....	.....	.....
6....	22	4.0	20.6	.257	.029	10.31	6.06	16.37	.58
7....	66	12.7	39.3	.....	.....	.....	.....	.....	.....
8....	66	12.8	35.9	.240	.019	30.72	6.64	37.36	.44
9....	220	17.5	42.9	.....	.....	.....	.....	.....	.....
10...	220	16.8	40.4	.335	.026	56.20	10.30	66.50	.24
SERIES 1B; SIXTY-DAY OATS HARVESTED ON JUNE 7, 1914[b]									
11...	0	0	6.0	.....	.....	.....	.....	.....	.....
12...	0	0	6.1	.....	.035	.....	2.14	2.14	.....
13...	11	4.7	10.0	.....	.....	.....	.....	.....	.....
14...	11	4.5	10.7	0.184	.038	8.28	4.11	12.38	.88
15...	22	7.6	13.6	.189	.032	14.36	4.35	18.72	.67
16...	22	7.2	14.0	.....	.....	.....	.....	.....	.....
17...	66	10.9	18.6	.....	.....	.....	.....	.....	.....
18...	66	12.1	15.4	.229	.038	27.71	5.85	33.56	.40
19...	220	16.8	22.9	.....	.....	.....	.....	.....	.....
20...	220	14.3	20.0	.354	.059	50.62	11.80	62.42	.22

[a] Seed planted in each pot in series 1A contained 0.46 per cent of phosphorus. Fifteen seeds contained 1.7 mgm. of phosphorus.

[b] Seed planted in each pot in series 1B contained 0.35 per cent of phosphorus. Fifteen seeds contained 1.29 mgm. of phosphorus.

TABLE III.—*Dry matter and phosphorus content of plant products from timothy and red-clover hay*

SERIES IE; TIMOTHY HARVESTED ON JULY 21, SEPT. 26, AND NOV. 25, 1914

Pot No.	Phosphate added.	Crop.			Phosphorus.					
		First cutting.	Second cutting.	Third cutting.	First cutting.	Second cutting.	First cutting.	Second cutting.	Total, two crops.	Percentage removed.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Per cent.*	*Mgm.*	*Per cent.*	*Mgm.*	*Mgm.*	
41	0	0.5	1.0	1.8	.....	.....	.....	.....	.....	.....
42	0	.8	.6	1.5	.....	.....	.....	.....	.....	.....
43	11	11.8	8.6	4.3	.....	.....	.....	.....	.....	.....
44	11	12.8	7.1	4.5	.....	.....	.....	.....	.....	.....
45	22	17.2	10.0	6.0	0.067	0.102	11.52	10.15	21.67	0.77
46	22	17.1	9.6	6.0	.....	.....	.....	.....	.....	.....
47	66	27.5	26.0	7.9	.....	.....	.....	.....	.....	.....
48	66	28.0	25.6	8.0	.....	.....	.....	.....	.....	.....
49	220	31.6	28.0	8.2	[a] .126	.170	37.80	49.98	87.78	.31
50	220	27.7	30.8	8.7	.....	.....	.....	.....	.....	.....

SERIES IH; RED-CLOVER HAY HARVESTED ON JULY 20, SEPT. 26, DEC. 25, 1914

71	0	.4	.2	.1	.....	.....	.....	.....	.....	.....
72	0	.3	.1	.1	.....	.....	.....	.....	.....	.....
73	11	5.1	3.5	3.4	.....	.....	.....	.....	.....	.....
74	11	4.0	2.8	3.5	.....	.....	.....	.....	.....	.....
75	22	7.8	12.2	8.4	.041	.055	3.23	6.73	9.967	.36
76	22	9.0	12.8	8.7	.....	.....	.....	.....	.....	.....
77	66	16.2	23.0	13.3	.....	.....	.....	.....	.....	.....
78	66	20.8	24.1	13.0	.....	.....	.....	.....	.....	.....
79	220	42.8	36.5	[b]10.0	.169	[c] .215	72.55	78.29	150.839	.54
80	220	54.5	37.8	14.2	.....	.....	.....	.....	.....	.....

[a] The phosphorus content of average timothy hay is 0.09 per cent.
[b] Attacked by worms.
[c] The phosphorus content of average red-clover hay is about 0.21 per cent.

TABLE IV.—*Dry matter and phosphorus content of plant products from cowpeas and soybeans*

SERIES IF; COWPEAS HARVESTED ON JULY 6, 1914

Pot No.	Phosphate added.	Grain.	Straw.	Phosphorus.					
				Grain.	Straw.	Grain.	Straw.	Total in grain and straw.	Percentage removed.
	Gm.	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Mgm.*	*Mgm.*	*Mgm.*	
51	0	0	2.8	.....	.....	.....	.....	.....	.....
52	0	0.3	2.7	.....	0.073	.....	1.97	1.97	.....
53	11	0.7	4.3	.....	.....	.....	.....	.....	.....
54	11	0	4.9	.....	.095	.....	4.66	4.66	.....
55	22	1.4	7.6	0.273	.097	3.82	7.37	11.19	0.40
56	22	0.7	6.7	.....	.....	.....	.....	.....	.....
57	66	11.7	23.8	.300	.117	35.08	27.92	62.99	.75
58	66	12.1	27.6	.....	.....	.....	.....	.....	.....
59	220	12.1	22.5	.....	.....	.....	.....	.....	.....
60	220	14.1	30.9	[a] .297	.128	41.86	39.40	81.26	.29

[a] Seed planted in each pot contained 0.434 per cent of phosphorus. Fifteen cowpea seeds contained 11.7 mgm. of phosphorus.

TABLE IV.—*Dry matter and phosphorus content of plant products from cowpeas and soybeans*—Continued.

SERIES 1G; SOYBEANS HARVESTED ON JUNE 10, 1914

Pot No.	Phosphate added.	Grain.	Straw.	Phosphorus.					
				Grain.	Straw.	Grain.	Straw.	Total in grain and straw.	Percentage removed.
	Gm.	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Mgm.*	*Mgm.*	*Mgm.*	
61	0	1.0	9.0	.....	.....	.....	.....	.....	.....
62	0	1.0	8.3	0.360	0.058	3.60	4.77	8.37	.....
63	11	2.0	9.2	.....	.....	.....	.....	.....	.....
64	11	2.8	10.2	.359	.045	10.06	4.54	14.60	1.04
65	22	3.5	13.5	.....	.....	.....	.....	.....	.....
66	22	2.4	10.8	.448	.062	10.76	6.64	17.40	.62
67	66	2.9	14.9	.....	.....	.....	.....	.....	.....
68	66	3.4	15.3	.449	.061	15.25	9.38	24.63	.29
69	220	4.7	15.2	.....	.....	.....	.....	.....	.....
70	220	4.2	13.4	[a] .448	.088	18.83	11.83	30.66	.11

[a] Seed planted in each pot contained 0.6 per cent of phosphorus. Fifteen soybean seeds contained 11.9 mgm. of phosphorus.

TABLE V.—*Dry matter and phosphorus content of plant products from alfalfa harvested on June 4, July 18, Sept. 26, and Nov. 11, 1914—series 1I*

Pot No.	Phosphate added.	First cutting.	Second cutting.	Third cutting.	Fourth cutting.	Phosphorus.[a]							
						First cutting.	Second cutting.	Third cutting.	First cutting.	Second cutting.	Third cutting.	Total, three cuttings.	Percentage removed from pots.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*	*Per ct.*	*Per ct.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Per ct.*
81	0	0.3	0.2	0.5	0.4	.....	.....	.....	.....	.....	.....	.....	.....
82	0	.3	.2	.6	.4	.....	.....	.....	.....	.....	.....	.....	.....
83	11	5.5	8.9	13.0	6.0	.....	.....	.....	.....	.....	.....	.....	.....
84	11	7.1	9.0	12.7	6.5	0.17	0.19	0.18	22	25	22	69.9	2.49
85	22	13.0	13.0	12.5	5.0	.....	.....	.....	.....	.....	.....	.....	.....
86	22	11.0	11.0	[b] 10.0	5.5	.....	.....	.....	.....	.....	.....	.....	.....
87	66	13.6	13.9	18.6	10.2	.....	.....	.....	.....	.....	.....	.....	.....
88	66	13.6	12.8	10.5	10.1	.....	.....	.....	.....	.....	.....	.....	.....
89	220	18.6	19.9	20.0	10.7	.10	.26	.28	17	51	55	124.6	.44
90	220	17.1	15.6	16.0	10.0	.....	.....	.....	.....	.....	.....	.....	.....

[a] Alfalfa hay contains 0.0172 per cent of phosphorus. [b] Attacked by worms.

TABLE VI.—*Dry matter produced by spring rye and barley—series 1C*

Pot No.	Rye.			Pot No.	Barley.		
	Phosphate added.	Grain.	Straw.		Phosphate added.	Grain.	Straw.
	Gm.	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*
21	0	0	1.9	31	0	0	8.7
22	0	0	1.8	32	0	0	7.2
23	11	0	10.9	33	11	2.6	20.3
24	11	0	11.6	34	11	1.3	16.4
25	22	0	22.2	35	22	2.0	17.8
26	22	0	21.0	36	22	8.5	22.3
27	66	0	37.0	37	66	5.0	23.3
28	66	0	38.2	38	66	14.2	23.4
29	220	0	45.1	39	220	20.9	34.8
30	220	0	42.0	40	220	16.5	28.0

Probably the most striking point shown by Tables II to VI is the gradual increase in the yield of both grain and straw from wheat, oats, and barley and in the hay from rye and timothy. In all cases larger applications of phosphorus gave higher returns, though not always in the same degree.

The grain yield of wheat is especially interesting. Eleven gm. of Tennessee brown rock phosphate produced 1.2 gm. of grain, while double this application produced 4.05 gm., or almost four times the yields from the light-application pots. Pots 7 and 8, which received 6 times as much phosphorus as pots 3 and 4, produced approximately 11 times as much wheat. Pots 9 and 10 received 20 times as much phosphorus as pots 3 and 4, but gave in return only about 14 times as much grain. Scarcely more evidence is necessary to show that wheat is able to take its phosphorus supply from Tennessee brown rock phosphate. It is also evident that the rate of yield is to a certain degree dependent upon the rate of application of the fertilizer. In the case of the heavy application, there were indications that the size of the pot was a limiting factor.

Oats responded more uniformly to the phosphate application than did wheat. The average yield of grain for pots 13 and 14 was 4.6 gm.; pots 15 and 16, which received double the quantity of phosphorus supplied to pots 13 and 14, yielded less than twice the amount of grain. For the highest application there is still a larger difference in the phosphorus applied and the crop produced, due, no doubt, to the limited size of the pot. The yield of straw followed about the same rate of increase as the grain.

Spring rye was not able to endure the heat of the summer days, and at the time of harvest growth had almost ceased without producing a single grain. The hay yield shows a gradual increase in dry matter as the application of phosphate rock was increased.

The yields from barley are not so consistent as those reported for wheat and oats. However, in all probability the same uniformity would have resulted had the crop not been attacked by smut. Although pots 34, 35, 37, 38, and 39 were badly affected, there was a gradual increase in grain and straw as the application of phosphorus increased. A yield of even 18 bushels for barley is not altogether unsatisfactory.

The data on timothy are no less interesting than those on the growth of the cereals, because of the opportunity to study the yields of the various cuttings. Timothy displays the same tendency to produce larger returns for greater quantities of phosphorus applied to the sand. For each pot there was a gradual decrease from the first to the last cutting, although the drop was less abrupt between the first and second than between the third and fourth cuttings.

Contrary to what might be expected the legumes respond to phosphate treatment no better than do the cereals. Perhaps on the whole this latter group produced larger gains than the former.

The results from the cowpeas show some points of particular interest. There was scarcely any seed produced for the pots to which 11 and 22 gm. of raw rock had been applied, but there was a decided increase for the pots which received 66-gm. applications. The next treatment, which was 220 gm. per pot, showed a slight increase, approximately 3 bushels per acre. For the cowpea hay the results are very similar to the seed yield. There is not a very marked increase in the hay production until the larger applications are made. The pots which received 66 gm. produced nearly as much hay as the pots which received 220 gm. of rock phosphate.

Cowpeas do not give results that correspond with those from soybeans. In the first place, the no-treatment pots produced a significant quantity of soy-bean seed, the yield on the acre basis amounting to 2.64 bushels, while the returns from the pots receiving the largest application just about quadrupled those from the former. The ratios for the yields of hay are about the same as for the grain. The yields for both seed and hay in the case of soybeans are unsatisfactory, which is not true of the cowpeas. It would seem that the latter legume utilizes rock phosphate better than soybeans.

To the practical agriculturist the returns from red clover will prove of considerable interest. It will be observed that the lowest treatment, 11 gm. per pot, produced hay at the rate of 772 pounds per acre. With double the application a little less than the former yield is recorded. When the lowest application is increased to six times the original amount, the yield of hay is increased about three times. The largest application, which was 20 times that of the lowest, produced practically 10 times as much hay as the first treatment. The above figures are for the first cutting only.

For the second harvest the relative yields of the 22- and 66-gm. treatments are more satisfactory than for the first cutting. It will be observed that the yield of the pots with 11-gm. applications and those with the 220-gm. applications hold the same relation for the second cutting as for the first. No direct comparison for the third cutting should be made, because pot 79, just previous to cutting, was attacked during a single night by a large cutworm which did considerable damage to the growing crop. It is true, however, that there had not been as much difference in the growth on the high-treatment pot as had been observed earlier in the season. The total yield for three cuttings for the heaviest application is large, but it can hardly be said that the pots which received 22 gm. of rock phosphate produced unprofitable yields.

Because of its extensive root system alfalfa would be expected to produce greater yields than clover. However, the difference in this experiment is not so marked. From four cuttings of alfalfa the yield of hay from the lowest treatment was 5,451 pounds, as against 1,819 pounds

of clover from the same treatment for three cuttings. For the next higher treatment the comparison is 6,426 pounds of alfalfa to 4,674 pounds of clover. The yields are approximately the same for the third application, but for the heavy treatment the clover almost doubles the yield from the alfalfa. Special attention is called to Plates LXXIII, LXXIV, and LXXV.

In drawing conclusions from an investigation of this kind the actual growth of the plant must be regarded as a most significant factor. However, an analytical study of the crops harvested can not fail to be of great value. Since phosphorus is the element with which this paper chiefly concerns itself, quantitative determinations were confined to that substance.

The determinations show that in practically all cases phosphorus is the limiting element in production. In every instance the dry matter increased as the phosphorus content of the pot was increased; also the quantity of phosphorus assimilated increased as the dry matter increased. The percentage of phosphorus in the plant in the majority of cases increased as the application of raw rock grew larger. This is especially noticeable in the hay crop. The most notable exceptions were observed in wheat and oat straw. There is no definite relation in the quantity of phosphorus applied and the percentage assimilated by the crop. There was a slight tendency in the grain for the percentage removed to decrease as the application was increased, but for the legumes this ratio does not hold. As high as 2.49 per cent of the phosphorus supplied in raw rock phosphate was removed in one season's growth of alfalfa.

COMPARATIVE STUDY OF THE PRODUCTIVE POWERS OF SIX MINERAL PHOSPHATES

The results from Tennessee brown rock phosphate proved so interesting that it was planned to determine the comparative value of mineral phosphates from the various mines of America. For this purpose Tennessee brown rock phosphate, Tennessee blue rock phosphate, South Carolina land rock phosphate, Utah rock phosphate, Canadian apatite, and Florida soft rock phosphate were selected.

The materials were ground so that all particles would pass through a sieve with 100 meshes to the inch and were applied in quantities which contained equal amounts of phosphorus for a given set of pots. Clover, oats, and cowpeas were grown with these different phosphates.

Because of limited space the small battery jars into which could be placed conveniently 4,800 gm. were selected for this rather extensive trial. Without crowding, eight plants per pot could be grown (Pl. LXXVI). Table VII gives the quantity of the phosphate applied and the yields of the crops in question. The planting was done on October 3, 1914, and the crops of clover were harvested on March 5 and April 9, 1915, while the oats were cut on February 5, 1915.

TABLE VII.—*Dry matter produced by different kinds of mineral phosphates—series 2*

Kind of phosphate added.	Red clover.				Sixty-Day oats.		
	Pot No.	Quantity of phosphate.	First cutting.	Second cutting.	Pot No.	Phosphate added.	Yield of straw.
		Gm.	*Gm.*	*Gm.*		*Gm.*	*Gm.*
None	1	0	0	0	39	0	1.1
Do	2	0	0	0	40	0	1.0
Tennessee brown rock	3	1.81	2.7	1.9	41	1.81	4.4
Do	4	1.81	1.0	.5	42	1.81	3.3
Do	5	3.62	4.3	3.3	43	3.62	5.7
Do	6	3.62	4.3	3.8	44	3.62	4.2
Do	7	10.86	7.1	4.8	45	10.86	6.9
Do	8	10.86	6.1	4.9	46	10.86	6.7
Canadian apatite	9	1.81	0	0	47	1.81	1.1
Do	10	1.81	0	0	48	1.81	1.1
Do	11	3.62	0	0	49	3.62	1.1
Do	12	3.62	0	0	50	3.62	1.3
Do	13	10.86	0	0	51	10.86	2.3
Do	14	10.86	0	0	52	10.86	1.4
South Carolina land rock	15	1.68	.2	1.3	53	1.68	2.2
Do	16	1.68	3.3	3.8	54	1.68	2.0
Do	17	3.36	.9	.8	55	3.36	1.3
Do	18	3.36	1.2	1.8	56	3.36	2.2
Do	19	10.07	1.1	0	57	10.07	3.4
Do	20	10.07	0	0	58	10.07	2.0
Utah rock	21	1.67	1.8	4.9	59	1.67	2.0
Do	22	1.67	3.4	5.0	60	1.67	1.4
Do	23	3.34	3.3	3.0	61	3.38	1.2
Do	24	3.34	0	0	62	3.38	1.9
Do	25	10.01	2.2	3.7	63	10.01	1.2
Do	26	10.01	2.9	4.7	64	10.01	1.5
Tennessee blue rock	27	1.72	0	0	65	1.72	1.6
Do	28	1.72	0	0	66	1.72	1.4
Do	29	3.44	.9	1.5	67	3.44	3.0
Do	30	3.44	.3	1.8	68	3.44	3.3
Do	31	10.33	7.0	6.7	69	10.33	3.3
Do	32	10.33	5.6	7.6	70	10.33	3.4
Florida soft rock	33	1.65	5.0	5.1	71	1.65	1.5
Do	34	1.65	3.5	5.1	72	1.65	1.2
Do	35	3.30	6.1	5.0	73	3.30	1.9
Do	36	3.30	4.4	5.9	74	3.30	1.9
Do	37	9.90	5.8	7.0	75	9.90	3.0
Do	38	9.90	5.8	6.9	76	9.90	3.2

In the foregoing series the greatest contrast is shown by the clover in its response to Tennessee brown rock phosphate and Canadian apatite. With brown rock the yield advanced rapidly with each increase in the amount of phosphate applied; but apatite, even with repeated plantings, failed to produce growth. South Carolina land rock phosphate proved better than apatite, but the growth for this treatment was very irregular. Utah phosphate excelled the South Carolina land rock phosphate. Except for

the lowest treatment, Tennessee blue phosphate gave fairly satisfactory yields. Florida phosphate for the three treatments gave almost as good returns as the Tennessee brown rock. Attention is called to the comparative yields of the Florida rock for the lowest and highest treatments. In this case a smaller quantity of the soft phosphate gave almost as large returns as the greater supply.

TABLE VIII.—*Dry matter produced by different kinds of mineral phosphate in red clover and Sixty-Day oats—series 3*

Kind of phosphate added.	Red clover.			Sixty-Day oats.			
	Pot No.	Phosphate.	Yield of hay.	Pot No.	Phosphate.	Grain.	Straw.
		Gm.	*Gm.*		*Gm.*	*Gm.*	*Gm.*
None	1	0	0.1	43	0	0.1	1.1
Do	2	0	.1	44	0	.1	1.1
Tennessee brown rock	3	11	1.0	45	11	.2	5.3
Do	4	11	1.0	46	11	1.4	5.4
Do	5	22	2.0	47	22	3.2	9.5
Do	6	22	2.0	48	22	3.0	8.2
Do	7	66	3.9	49	66	5.3	10.3
Do	8	66	4.1	50	66	5.2	9.0
Do	9	220	3.8	51	220	3.8	13.0
Do	10	220	4.0	52	220	5.0	12.0
Canadian apatite	11	11	.1	53	11	0	1.4
Do	12	11	.1	54	11	0	1.4
Do	13	22	.2	55	22	0	1.4
Do	14	22	.2	56	22	0	1.6
Do	15	66	.1	57	66	0	1.3
Do	16	66	.3	58	66	0	1.0
Do	17	220	.1	59	220	0	1.2
Do	18	220	.1	60	220	0	1.5
Utah rock	19	10.11	.2	61	10.11	0	.8
Do	20	10.11	.2	62	10.11	0	.8
Do	21	20.22	.7	63	20.22	0	.8
Do	22	20.22	.5	64	20.22	0	1.3
Do	23	60.66	.5	65	60.66	0	.9
Do	24	60.66	.4	66	60.66	0	.9
Do	25	202.20	.2	67	202.20	0	.9
Do	26	202.20	.1	68	202.20	0	.9
South Carolina land rock	27	10.16	.1	69	10.16	0	1.0
Do	28	10.16	.1	70	10.16	0	.8
Do	29	20.32	.1	71	20.32	0	.9
Do	30	20.32	.1	72	20.32	0	.9
Do	31	60.96	.1	73	60.96	0	.7
Do	32	60.96	.1	74	60.96	0	.8
Do	33	203.20	.1	75	203.20	0	.6
Do	34	203.20	.1	76	203.20	0	.5
Tennessee blue rock	35	10.42	.2	77	10.42	0	.9
Do	36	10.42	.2	78	10.42	0	1.0
Do	37	20.84	.3	79	20.84	0	.9
Do	38	20.84	.1	80	20.84	0	.7
Do	39	62.52	.4	81	62.52	0	.8
Do	40	62.52	.5	82	62.52	0	.9
Do	41	208.40	.3	83	208.40	0	1.0
Do	42	208.40	.0	84	208.40	0	.9

Under greenhouse conditions it was extremely difficult to secure a seed crop of oats during the winter months; hence, the differences of productive power of the various phosphates must be measured by the yields of straw. In a general way the results obtained in this manner are in harmony with those reported for clover. The brown rock excelled the other phosphates in the production of hay; blue phosphate ranks second; and where apatite was applied it will be observed that the plants made very little growth. Plate LXXVI indicates greater difference in the growth of clover than the dry weight of the top.

The above data indicate that there was an increase in yield as the quantity of phosphorus was increased. The question naturally arises as to the point at which larger applications of rock phosphate failed to produce greater returns. In order to answer this query, the following results are inserted (Table VIII): The lowest treatment in the table is about the same as the highest application in series two. This set of pot cultures was planted on August 27, 1914, and harvested on December 4, 1914.

By referring to the clover in Table VIII, a comparison of the yields shows nothing particularly in favor of excessive quantities of rock phosphate. One point, however, is of interest, and that is that the oats produced a seed crop on the land with the heavy application of brown rock. The hay on the other pots was scarcely more than could be produced by the phosphorus in the seeds planted.

TABLE IX.—*Dry matter produced by various kinds of mineral phosphates in cowpeas, series 3*

Kind of phosphate added.	Pot No.	Phosphate.	Yield of hay.	Kind of phosphate added.	Pot No.	Phosphate.	Yield of hay.
		Gm.	*Gm.*			*Gm.*	*Gm.*
None	1	0	1.0	Utah rock	22	20.22	2.2
Do	2	0	0.9	Do	23	60.66	1.4
Tennessee brown rock	3	11	1.1	Do	24	60.66	1.4
Do	4	11	1.9	Do	25	202.20	.9
Do	5	22	4.6	Do	26	202.20	.9
Do	6	22	4.5	South Carolina land rock	27	10.16	2.0
Do	7	66	11.9	Do	28	10.16	2.2
Do	8	66	10.7	Do	29	20.32	6.4
Do	9	220	17.4	Do	30	20.32	5.4
Do	10	220	13.9	Do	31	60.96	4.7
Canadian apatite	11	11	1.0	Do	32	60.96	4.5
Do	12	11	1.0	Do	33	203.20	.9
Do	13	22	1.0	Do	34	203.20	1.5
Do	14	22	1.0	Tennessee blue rock	35	10.42	4.0
Do	15	66	1.6	Do	36	10.42	3.5
Do	16	66	1.3	Do	37	20.84	4.7
Do	17	220	.9	Do	38	20.84	5.0
Do	18	220	.9	Do	39	62.52	3.9
Utah rock	19	10.11	2.0	Do	40	62.52	4.2
Do	20	10.11	2.9	Do	41	208.40	1.9
Do	21	20.22	3.4	Do	42	208.40	2.1

Soon after the clover was harvested in series 3, these pots were seeded to cowpeas. Cowpeas were planted to determine the ability of this legume to utilize the phosphorus contained in mineral phosphates. The cultures were seeded January 24, 1914, and harvested April 5, 1914 (Table IX).

The results secured for series 4 are in accord with those from the clover and oats grown on the pots with large applications. The pots to which had been added brown rock phosphate produced a good return of cowpea hay after having given satisfactory yields of clover.

The data presented in the previous tables show conclusively that certain species of plants have the power to obtain phosphorus from brown rock phosphate, but how they acquire this element is the problem of vital concern. Do they secure their phosphorus without indirect aid and what influence do other plant foods applied in a soluble form exert on the phosphorus compounds?

It will be remembered that the sand cultures were maintained at a moisture content of 14 per cent. The plant food application, the infusion, and the water added when the seeds were planted constituted the first moisture supply; or, in other words, all these solutions brought the water content up to 14 per cent. In most of the cases five applications of plant food were sufficient to produce a crop of clover or oats.

To estimate the influence of water and plant-food solutions on the solubility of the phosphates, quantities of raw rock which correspond to the smallest application (1.81 gm.), soluble plant food equivalent to five applications, and water sufficient to bring the supply of the solution to the same amount that was necessary to bring the moisture content to 14 per cent, or 672 c. c., were placed in a 1-liter flask and shaken each day for three months. The soluble phosphorus was then determined with the results shown in Table X.

TABLE X.—*The influence of soluble plant foods on the solubility of the phosphorus in mineral phosphates*

Material applied and pot No.	Kind of phosphate.	Quantity of phosphate.	Phosphorus dissolved.
Water only:		*Gm.*	*Mgm.*
1	Tennessee brown rock	1.81	0.25
Water and soluble plant food:			
2	do	1.81	.33
3	Tennessee blue rock	1.72	.05
4	Canadian apatite	1.81	.05
5	South Carolina land rock	1.68	.056
6	Utah rock	1.67	.14
7	Florida soft rock	1.65	.28

The solutions dissolve very little of the phosphorus from the insoluble phosphate.

Brown rock phosphate and Florida soft rock phosphate gave the best results with clover, but the former was very much better suited for oats than the latter. There is a slight indication that phosphates which are more soluble in water are more easily assimilated by plants.

THE INFLUENCE OF FERMENTING DEXTROSE AND CROP RESIDUES ON THE AVAILABILITY OF PHOSPHORUS IN FINELY GROUND ROCK PHOSPHATE

Though the data are not conclusive, a large number of field experiments conducted in America show that raw phosphate, when applied in conjunction with organic matter, produces very appreciable increases in crop yields. The work which follows is an effort to determine the influence of decaying substances on the availability of the phosphorus in crude phosphate rock. Dextrose was employed because it ferments rapidly under greenhouse conditions. Crop residues are also included in this section, but owing to the slow growth of crops through the winter months it will not be possible to do more than to make a preliminary report on this phase of the problem.

Throughout the study included in this division, the glass battery jars were utilized with success and the same quantity of sand employed as previously noted—namely, 4,800 gm. per pot. For all the cultures grown in the dextrose section, the sand was leached with dilute hydrochloric acid.

The first series reported below was outlined primarily to secure data on the value of rock phosphate alone and in conjunction with dextrose for rye and clover. It will be observed that the applications of the rock phosphate and the dextrose were made on the percentage basis. In order to hasten fermentation, an infusion from a rich soil was a part of the treatment. This series was planted on April 12, 1913, and harvested on August 19, 1913.

Since dextrose applied at the rate of 48 gm. per pot injured the rye and destroyed the clover, a point of importance to decide was what quantity would not injure plant development, but would assist in the liberation of phosphorus. With this point in mind, series 6 was planned. The planting was done on June 21, 1913, and the crop harvested on December 1, 1913. (See Table XI.)

The dextrose in series 5 had no beneficial influence. If the average of pots 7, 8, and 9 is compared with the results from either set of pots 1, 2, and 3 or pots 4, 5, and 6, it will be evident that the dextrose is harmful. Clover failed to make growth where the dextrose was added, but did fairly well on the pots which received rock phosphate alone.

The data in Table XI show that dextrose fails to be of any particular advantage for rendering phosphorus available for the growth of rye and clover. Even small quantities of this material killed clover.

TABLE XI.—*Dry matter produced by Tennessee brown rock phosphate and dextrose in growing spring rye and red clover*

SERIES 5

Rye.					Red clover.				
Pot No.	Phosphate added.	Dextrose added.	Infusion added.	Hay yield.	Pot No.	Phosphate added.	Dextrose added.	Infusion added	Hay yield.
	Gm.	*Gm.*	*C. c.*	*Gm.*		*Gm.*	*Gm.*	*C. c.*	*Gm.*
1.....	48	48	0	22.9	19...	48	48	20	[a] 0
2.....	48	48	0	33.9	20...	48	48	20	[a] 0
3.....	48	48	0	31.3	21...	48	48	20	[a] 0
4.....	48	48	20	35.3	22...	48	0	0	3.0
5.....	48	48	20	32.3	23...	48	0	0	3.8
6.....	48	48	20	22.0	24...	48	0	0	2.9
7.....	48	0	0	27.2					
8.....	48	0	0	32.5					
9.....	48	0	0	36.0					

SERIES 6

3....	48	4.8	20	14.6	17...	48	4.8	20	[b] 20
4....	48	4.8	20	17.9	18...	48	4.8	20	[b] 20
5....	48	14.4	20	17.5	19...	48	14.4	20	[b] 20
6....	48	14.4	20	16.0	20...	48	14.4	20	[b] 20
7....	48	24.0	20	16.8	21...	48	24.0	20	[b] 20
8....	48	24.0	20	20.7	22...	48	24.0	20	[b] 20
9....	48	48.0	20	14.5	23...	48	48.0	20	[b] 20
10....	48	48.0	20	11.6	24...	48	48.0	20	[b] 20
11....	48	0	20	18.7	25...	48	0	20	3.0
12....	48	0	20	17.7	26...	48	0	20	2.9
					27...	0	0	20	0
					28...	0	0	20	0

[a] The clover in pots 19, 20, and 21 was dead on June 29, 1913.
[b] The clover in pots 17 to 24, inclusive, was dead in less than 1 month after planting.

Rye and clover were replaced in series 7 (Table XII) by cowpeas, with the feeling that the latter crop might respond more readily to various treatments (Pl. LXXVII). The cowpeas were planted on July 4, 1913, and harvested on October 2, 1913.

The cowpeas grown in series 7 show clearly that so small a quantity of dextrose as 4.8 per cent was injurious to plant growth. Where dextrose was applied, smaller quantities of phosphorus were assimilated, due, no doubt, to the injury of the plant by the acids formed from decomposing dextrose. However, the percentage of phosphorus increased as the quantity of the fermentable substance was increased.

TABLE XII.—*Dry matter and phosphorus content of plant products of cowpeas from pot cultures, with the addition of Tennessee brown rock phosphate and dextrose; series 7*

Pot No.	Phosphate added.	Dextrose added.	Infusion added.	Hay yield.	Phosphorus content.		
					Hay.	Hay yield.	Removed from pot.
	Gm.	*Gm.*	*C. c.*	*Gm.*	*Per cent.*	*Mgm.*	*Per cent.*
1	48	4.8	20	25.0	0.319	79.75	1.30
2	48	4.8	20	22.3	.....	.....	.....
3	48	14.4	20	11.9	.....	.....	.....
4	48	14.4	20	8.8	.381	33.33	.54
5	48	24.0	20	[a] 0	.....	.....	.....
6	48	24.0	20	[a] 0	.....	.....	.....
7	48	48.0	20	[a] 0	.....	.....	.....
8	48	48.0	20	[a] 0	.....	.....	.....
9	0	48.0	20	[a] 0	.....	.....	.....
10	0	48.0	20	[a] 0	.....	.....	.....
11	48	0	20	29.9	.....	.....	.....
12	48	0	20	29.1	.286	85.51	1.40
13	0	0	20	4.0	.....	.....	.....
14	0	0	20	3.0	[b].128	3.84	.....

[a] The plants on pots 5 to 10, inclusive, were all dead by Aug. 9, 1913.
[b] Five cowpea seeds were planted in each pot. These contained 3.92 mgm. of phosphorus.

Rye, clover, and cowpeas failed to thrive wherever the smallest quantity of dextrose was present. There is but little doubt that this destructive influence is due to the decomposition of dextrose. If this conclusion be true, a liberal use of calcium carbonate should neutralize the acids developed, and a normal growth of the plants should result. Series 8 (Table XIII) was designed for determining what influence calcium carbonate would have in stimulating plant growth by producing an alkaline medium and to ascertain whether calcium served as a food.

TABLE XIII.—*Dry matter produced in spring rye by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 8*

Pot No.	Phosphate added.	Dextrose added.[a]	Calcium carbonate added.	Hay yield.	Pot No.	Phosphate added.	Dextrose added.[a]	Calcium carbonate added.	Hay yield.
	Gm.	*Gm.*	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	48	48	10	7.2	13	0	0	0	0.5
2	48	48	10	9.3	14	0	0	0	.6
3	48	48	0	5.2	15	48	0	0	11.2
4	48	48	0	3.5	16	48	0	0	12.1
5	48	48	10	7.0	17	48	4.8	10	10.7
6	48	48	10	7.0	18	48	4.8	10	10.0
7	0	0	0	.1	19	48	4.8	0	9.6
8	0	0	0	.1	20	48	4.8	0	9.0
9	48	48	0	7.8	21	0	0	0	.4
10	48	48	0	8.9	22	0	0	0	.3
11	0	0	0	.1	23	48	48	0	6.5
12	0	0	0	.1	24	48	48	0	6.0

[a] Pots 5 and 6 were leached and the leachings placed on pots 7 and 8. Pots 9 and 10 were leached and the leachings placed on pots 11 and 12. Pots 13 and 14 received all plant food but phosphorus. Pots 21 and 22 received nothing. Pots 23 and 24 were leached and drainage water taken for analytical purposes.

Series 8 shows that dextrose in conjunction with calcium carbonate did not give as good results as raw rock phosphate alone, and that 10 gm. of calcium carbonate was not sufficient to nullify the harmful influence of the dextrose.

Series 9 (Table XIV), which follows, is just the same as series 8 except that cowpeas are substituted for rye, the object being to determine the relative response of rye and cowpeas to the different treatments.

TABLE XIV.—*Dry matter produced in cowpeas by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 9*

Pot No.	Phosphate added.	Dextrose added.	Calcium carbonate added.	Hay yield.	Pot No.	Phosphate added.	Dextrose added.	Calcium carbonate added.	Hay yield.
	Gm.	*Gm.*	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
1F..	48	48	10	5.5	13F..	0	0	0	3.3
2F..	48	48	10	5.3	14F..	0	0	0	3.0
3F..	48	48	0	5.2	15F..	48	0	0	12.4
4F..	48	48	0	4.0	16F..	48	0	0	14.5
5F..	48	[a] 48	10	5.3	17F..	48	4.8	10	8.1
6F..	48	48	10	6.1	18F..	48	4.8	10	10.1
7F..	0	0	0	3.8	19F..	48	4.8	0	11.1
8F..	0	0	0	3.8	20F..	48	4.8	0	11.8
9F..	48	48	0	4.2	21F..	0	0	0	3.9
10F..	48	48	0	3.0	22F..	0	0	0	4.5
11F..	0	0	0	3.1	23F..	48	48	0	4.0
12F..	0	0	0	2.3	24F..	48	48	0	4.0

[a] See note to Table XIII.

Series 9 shows that brown rock phosphate, dextrose, and a limited supply of calcium carbonate failed to give as good results with cowpeas as raw phosphate alone. For further comparison see Plate LXXVIII.

Thus far it has not seemed necessary to use calcium carbonate alone, because it was thought that the plants would get enough calcium, for full growth from the phosphate, however, in order to avoid criticism at this point calcium carbonate was added to certain pots in the following series. The quantity of this compound was increased to 48 gm. per pot, which is almost five times as much as the application in the preceding series.

By making a comparison of the pots which received raw rock phosphate alone and those which received raw rock and calcium carbonate very little difference in the yield is observed, only 0.6 gm. more in favor of the addition of the lime compound. There is no strong evidence in Table XV to show that the omission of calcium was a mistake. Where lime was applied with rock phosphate and dextrose, the injury by dextrose reported earlier was nullified by the application of lime (Pl. LXXIX and LXXX).

Attention is called to the percentage of phosphorus in the cowpea hay grown in the pots which received soluble phosphorus.

TABLE XV.—*Dry matter produced in cowpeas by Tennessee brown rock phosphate with the addition of dextrose and calcium carbonate—series 10*

Pot No.	Phosphate added.	Dextrose added.	Calcium added.	Hay yield.	Phosphate content.		
					Hay.		Percentage removed from pot.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Per cent.*	*Mgm.*	
1G...	0	0	10	3.0	0.111	3.37	.....
2G...	0	0	10	3.5	.....	.....	.....
3G...	0	0	0	2.8	.....	.....	.....
4G...	0	0	0	3.0	.119	3.57	.....
5G...	48	0	0	6.1	.....	.....	.....
6G...	48	0	0	6.3	.246	15.55	0.25
7G...	48	0	0	5.3	.....	.....	.....
8G...	48	0	0	6.8	.236	16.05	.52
9G...	48	0	10	7.4	.156	11.52	.19
10G...	48	0	10	6.2	.239	11.71	.19
11G...	48	48	0	4.9	.....	.....	.....
12G...	48	48	0	4.6	.....	.....	.....
13G...	0	48	48	3.2	.054	1.73	.....
14G...	0	48	48	3.3	.....	.....	.....
15G...	48	48	10	6.8	.....	.....	.....
16G...	48	48	10	7.0	.122	8.54	.14
17G...	(*a*)	0	0	4.9	.....	.....	.....
18G...	(*a*)	0	0	4.8	.660	31.68	.....
19G...	0	0	0	3.0	.....	.....	.....
20G...	0	0	0	3.0	.104	5.19	.....
21G...	*b* 48	0	0	7.0	.....	.....	.....
22G...	48	0	0	6.8	.388	24.06	.39
23G...	48	48	48	6.9	.....	.....	.....
24G...	48	48	48	6.3	.115	7.25	.12

a Soluble phosphate.
b In pots 21 and 22 potassium chlorid was substituted for potassium sulphate.

Under the conditions of this experiment, fermenting dextrose was a failure in bringing about the liberation of phosphorus. Since the use of crop residues is a common farm practice for supplying organic matter, which is said to aid in the liberation of phosphorus, the series next reported was planned with timothy hay and clover substitutes for dextrose.

Timothy and clover cultures on which data are reported in Table III are used for this phase of the problem. Of the duplicate pots the hay from one was taken for analytical study, while the product of the other was ground and returned as organic matter. This series (Table XVI) shows the original treatment with the quantity of air-dried hay turned under. The contents of the pots to which organic matter was added were turned out and the ground material thoroughly incorporated with the sand on December 3, 1914. On January 23, 1915, the pots were planted to the respective crops. They were harvested on April 17, 1915.

The organic matter with phosphate in the above series gave larger returns in most cases than where the phosphate was alone. This increase is probably due to the liberation of phosphorus by the decaying residues or the organic phosphorus in the crop residues themselves.

TABLE XVI.—*Dry matter produced in timothy and red clover by Tennessee brown rock phosphate and crop residues—series 11*

Timothy.				Red clover.			
Pot No.	Phosphate added.	Organic matter added.	Hay yield.	Pot No.	Phosphate added.	Organic matter added.	Hay yield.
	Gm.	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*
41[a]	0	0	0.25	71	0	0	0.02
42	0	2.9	.05	72	0	.55	.10
43	11	0	.02	73	11	0	.40
44	11	24.4	.20	74	11	10.5	.10
45	22	0	.30	75	22	0	2.70
46	22	32.7	1.10	76	22	30.5	6.40
47	66	0	3.40	77	66	0	4.70
48	66	61.6	10.70	78	66	57.9	12.50
49	220	0	8.70				
50	220	67.2	11.70				

[a] See series 1, Tables II to VI.

INFLUENCE OF SIZE OF PARTICLES ON THE AVAILABILITY OF PHOSPHORUS IN MINERAL PHOSPHATES

The degree of fineness of rock phosphate particles has been held by many investigators to be an important factor in the availability of mineral phosphates. Dr. Jordan, of the New York Experiment Station, showed rather conclusively that plants supplied with very finely ground rock phosphate contained more phosphorus and produced a greater quantity of dry matter than those supplied with the coarser grades. For the purpose of determining a comparative value of the same rock when ground very fine to that left in particles of a larger size, series 12 (Table XVII) was begun. As a check on the rock which was obtained from the Mount Pleasant mills some lump rock from the same source was secured and ground. These results are reported along with the data on the influence of the size of particles on the availability.

TABLE XVII.—*Relation of size of phosphate particles to the availability of phosphorus by Sixty-Day oats harvested on July 10, 1915—series 12*

Pot No.	Phosphate added.	Fineness.	Grain yield.	Straw yield.	Pot No.	Phosphate added.	Fineness.	Grain yield.	Straw yield.
	Gm.		*Gm.*	*Gm.*		*Gm.*		*Gm.*	*Gm.*
1	0		0	1.20	10	2.6	200 degrees or over	4.00	6.3
2	0		0	1.60	17	2.6	80 to 100 degrees	5.90	6.80
5	2.6	80 to 100 degrees	3.25	6.15	18	2.6	do	7.00	10.05
6	2.6	do	2.05	5.35	19	2.6	100 to 200 degrees	5.80	11.05
7	2.6	100 to 200 degrees	3.70	6.60	20	2.6	do	7.70	11.35
8	2.6	do	4.70	9.30	21	2.6	200 degrees or over	8.65	11.10
9	2.6	200 degrees or over	5.00	8.70	22	2.6	do	7.15	13.20

Pots 5 to 10, inclusive, received the ground rock phosphate as it was obtained from the mills. The degree of fineness varied from that passing a sieve 80 to 100 meshes to the inch to that which would go through a sieve of 200 meshes to the inch. Pots 17 to 22, inclusive, received the ground phosphate which was shipped in the lump form and afterward ground to the same degree of fineness as that ground at the mill.

There is a tendency for the dry matter to increase as the degree of fineness increases. The phosphate received from the mill in lump form was slightly better than that sent to us in a ground condition.

DISCUSSION

Under the conditions of these experiments a fairly large portion of the phosphorus in brown rock phosphate was available for plant growth. The quantity was variable, depending upon the crops and the circumstances attending the full development of the plant. The data show only a very small amount of phosphorus soluble in water and plant food solutions. It is clear that other factors which might bring about availability must be considered. The sand cultures contained very little organic matter; hence, these slight fermentable substances should not be considered. There is nothing left but the plant for our examination and there is abundant proof that the plant itself is a significant item. Since plants excrete large quantities of carbonic acid, there is but little question that this substance plays the primary roll in the liberation of phosphorus.

The reactions with carbon dioxid which occur when tricalcium phosphate is put into sand cultures of the kind described in these pages may be shown in the following manner:

$$\text{(A)}\quad \underset{\text{Gaseous}}{CO_2} + H_2O \rightleftarrows \underset{(1)}{H_2CO_3} \rightleftarrows \underset{\text{In solution}}{H^+ + \overline{HCO_3}} \rightleftarrows \underset{(2)}{H^{++} + CO_3} \qquad (3)$$

$$\text{(B)}\quad \underset{\text{Solid}}{Ca_3(PO_4)_2} \rightleftarrows \underset{(4)}{Ca_3(PO_4)_2} \rightleftarrows \underset{\text{In solution.}}{3Ca^{++} + \overline{\overline{2PO_4}}} \qquad (5)$$

When A and B are mixed, the following equilibria develop:

$$\text{(C)}\quad \overline{\overline{PO_4}} + H^+ \underset{(6)}{\overline{HPO_4}} \text{ (The ion of reverted phosphate)}$$

$$\overline{\overline{HPO_4}} + H^+ \underset{(7)}{\rightleftarrows} H_2PO_4 \text{ (The ion of soluble phosphate)}$$

$$\overline{H_2PO_4} + H^+ \underset{(8)}{\rightleftarrows} H_3PO_4$$

Equations A and B make it evident that the hydrogen ion concentration for the various acids will determine the course of the reactions rendering the rock phosphate available. The hydrogen ion concentration is made up of two factors—namely, the concentration and the strength of the acid. Obviously under the conditions of these experiments saturated solutions of rock phosphate and carbonic acid are employed. The relative insolubility of the rock phosphate tends to decrease greatly the concentration of the H^+ from either 6, 7, or 8. The relatively greater solubility of the calcium bicarbonate, since it furnishes $\overline{HCO_3}$, would also tend to decrease the H^+ concentration from carbonic acid, but this factor of common ion effect is of far less importance upon the concentration of the H^+ from H_2CO_3 than the solubility of the tricalcium phosphate upon equations 6, 7, and 8, especially since the Ca^{++} from the $Ca(HCO_3)_2$ is removed by plants.

Assuming equivalent or unit concentrations of the substances H_2CO_3, H_3PO_4, $\overline{H_2PO_4}$, and $\overline{\overline{HPO_4}}$ are present—that is, eliminating the factor of concentration of the substances producing the H—the relative strength of these acids is given by their ionization constants, thus:

(1)[a] $H_2CO_3 \rightleftarrows H^+ + \overline{HCO_3}$ $\quad Ka^{18°}\ 3.0\times10^{-7}$

(6) $\overline{\overline{HPO_4}} \rightleftarrows \overline{\overline{PO_4}} + H^+$ $\quad Ka^{18°}\ 3.6\times10^{-13}$

(7) $\overline{H_2PO_4} \rightleftarrows \overline{\overline{HPO_4}} + H^+$ $\quad Ka^{18°}\ 1.95\times10^{-7}$

(8) $H_3PO_4 \rightleftarrows \overline{H_2PO_4} + H^+$ $\quad Ka^{18°}\ 1.1\times10^{-2}$

The mass law for monobasic acids (HAc) has the form $Ka = \frac{(\text{Conc } H^+)\ (\text{Conc Ac})}{\text{Conc HAc}}$. Since the acids of equations 1, 6, and 7 are weak acids ($Ka < 10^{-4}$), the mass law assumes the form $Ka = (\text{Conc } H^+)\ (\text{Conc Ac}) = (\text{Conc } H^+)^2$, because the concentration of HAc is practically unity. The concentrations of H^+ for these equations at 18° C. are for:

(1) $\sqrt{3\times10^{-7}} = 5.5\times10^{-4}$ for $C^{(1)}H^+$

(6) $\sqrt{3.6\times10^{-13}} = 6\times10^{-7}$ for $C^{(6)}H^+$

(7) $\sqrt{1.95\times10^{-7}} = 4.4\times10^{-4}$ for $C^{(7)}H^+$

For the first hydrogen of H_3PO_4 the above expression can not be used, since the amount H_3PO_4 compared to its ions is small rather than large. Here the mass law must be used in its true form, $K = \frac{C\alpha^2}{1-\alpha}$, where C is equal to the initial concentration of H_3PO_4 and α degrees of ioniza-

[a] See equations, page 508.

tion. For the purpose α is taken equal to 90 per cent, from which the concentration of H is calculated thus:

$$Ka^{18°} = \frac{C\alpha^2}{1-\alpha}, \quad \text{where} \quad (C) = Ka\frac{(1-\alpha)}{\alpha} = \text{concentration of H.}$$

$$\therefore \text{ concentration of } H^+(C^{(8)}H^+) = \frac{1.1 \times 10^{-2}(0.1)}{0.9} = \frac{1.1 \times 10^{-3}}{0.9} = 1 \times 10^{-3} =$$

0.001.

It is seen that only the first H of H_3PO_4 can furnish a greater concentration of H^+ than H_2CO_3 for equivalent concentrations. In the actual experiment the concentration of H_3PO_4 is much less than that of H_2CO_3. However, the availability of the rock phosphate by means of H_2CO_3 is not conditioned by the liberation of free H_3PO_4 according to equation 8. Equation 6 or 7 is driven in the direction to remove H^+, would render the tricalcium phosphate more available, but a reaction between ions proceeds if a lesser ionized product be formed. Calculations of the H^+ concentration for equations 1, 6, and 7 shows that for equivalent concentrations the H^+ from carbonic acid is greatly in excess of the H^+ concentration for equations 6 and 7. So if equations 1, 6, and 7 are present simultaneously $\overline{\overline{HPO_4}}$ and $\overline{H_2PO_4}$ of equations 6 and 7 would be formed by the union of H^+ of H_2CO_3 with $\overline{\overline{PO_4}}$ and $\overline{\overline{HPO_4}}$, respectively, thus causing more $Ca_3(PO_4)_2$ to dissolve to reestablish the equilibria for equations 6 and 7. It is a fact, however, that a greater concentration of H_2CO_3 is present than any of the ionizing substances, as $\overline{\overline{HPO_4}}$, $\overline{H_2PO_4}$, or H_3PO_4. This would increase the rate of availability of the tricalcium phosphate.

These calculations are borne out by the fact that more $Ca_3(PO_4)_2$ dissolved in water containing H_2CO_3 than in pure water. Seidel's solubility tables state that 1 liter of water saturated with H_2CO_3 dissolves 0.15 to 0.30 gm. of $Ca_3(PO_4)_2$ at 25°, while 1 liter of pure water dissolved only 0.01 to 0.10 gm. of $Ca_3(PO_4)_2$ at 25°.

Reactions 6 and 7 may be shown in the nonionic form as follows:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2H_2(PO_4)_2 + CaH_2(CO_3)_2 \text{ or}$$
$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2(HPO_4)_2 + Ca(HCO_3)_2$$
$$Ca_2H_2(PO_4)_2 + 2H_2CO_3 \rightleftarrows CaH_4(PO_4)_2 + CaH_2(CO_3)_2$$
$$\downarrow \qquad\qquad \downarrow$$
$$\text{or} \qquad\qquad \text{or}$$
$$Ca(H_2PO_4)_2 \qquad Ca(HCO_3)_2$$

In the first equation calcium is found in a form readily assimilated by plants, and in the second the monocalcium phosphate is in a very assimilable form. On this equation we have based our belief that there is no necessity for applying lime to sand cultures to which had previously been

added raw rock phosphate. When the calcium bicarbonate and monocalcium phosphate are both removed from the medium of growth by plants, the reaction is driven rapidly to the right. Mass relationship in a mixture of this kind confirms such an interpretation as the one presented above. Our first assumption, that plants should get their calcium from rock phosphate in the same manner that they get their phosphorus, is supported at several points in this work. This must be so, since the calcium is furnished by the calcium salt of phosphoric acid or by the bicarbonate. There was no greater growth when calcium carbonate was added than where raw rock alone was used. In fact, the growth might be even less, since calcium carbonate might furnish a greater concentration of $Ca(HCO_3)_2$ or HCO_3, which might decrease the concentration of H from equation 1, thus decreasing the rate of the availability of rock phosphate.

The most marked feature of the investigation is the difference of the availability of the various minerals. The fact that the crop yields increase as the application of the brown rock phosphate was increased indicates that a portion of the phosphorus was readily assimilated while the plants were young, and that by the time these plants became well established they were able to utilize the more insoluble form. If we are to assume that a part of the phosphorus is of animal origin, this position probably is more tenable, or on the other hand, through long years of weathering the compound had been so changed that a portion was more easily taken up by plants than before weathering began.

There is an indication that the crops grown first took up the more available phosphorus and that the second crop made very slow growth because the more soluble phosphorus was removed by the first crop and nothing left but the rather insoluble for later crops. These points have proof from the cowpeas on the large application series and the clover on the crop residue series.

Brown rock phosphate and Florida soft rock phosphate lead the others in supplying available phosphorus for plant nutrition, especially for clover. The brown rock phosphate leads for all the crops. These two phosphates gave the largest quantity of phosphorus soluble in water and plant-food solutions. The results indicate a relation in solubility in plant-food solution and the availability for plants.

The difference in the assimilation of these phosphates can not be attributed to the degree of fineness of the particles, since they were all ground, so that the entire sample passed through a sieve of 100 meshes to the inch. If the degree of fineness influenced the results, the differences then come from the size of particles, which were smaller than those found in commercial phosphates.

The variation in the agricultural value of the six mineral phosphates studied is difficult to explain. Their productive powers seemed not to

have any direct relation to the amount of phosphorus which they contained. Brown rock, which had the smallest amount of phosphorus, produced the most satisfactory yields. The differences must be attributed to modes of formation and weathering since the minerals were laid down.

SUMMARY

(1) Phosphorus in rock phosphate can be assimilated by farm crops in sand cultures under greenhouse conditions, even in the absence of decaying residues.

(2) Crop residues, when employed in conjunction with brown rock phosphates, were beneficial.

(3) Tennessee brown rock phosphate, Florida soft rock phosphate, and Tennessee blue rock phosphate in the heavier applications proved superior to South Carolina land rock phosphate, Utah rock phosphate, and Canadian apatite, for oats, clover, and cowpeas when grown in sand.

(4) The phosphorus in brown rock phosphate and Florida soft rock phosphate was more soluble in water and in plant-food solutions than the phosphorus in other mineral phosphates. The superiority of these two phosphates over the others tested is shown chiefly by the first crop.

(5) Chemical analysis showed that the plant-food solutions applied did not appreciably modify the results.

(6) The cereals produced as satisfactory yields as the legumes.

(7) The crop yields tended to increase as the application of rock phosphate increased up to a point where the size of the pots seemed to be a limiting factor, apatite being the only exception.

(8) The plants obtained their calcium, as well as their phosphorus, from brown rock phosphates. No better results were secured when calcium carbonate was applied than when rock phosphate alone was used.

(9) There was no particular relation between the citric-acid-soluble phosphorus and the availability of these phosphates for plants.

(10) Dextrose, when used as a fermentable substance, was harmful.

(11) The degree of fineness is a factor which determines to some extent the availability of rock phosphate, as indicated by the brown rock.

(12) These investigations extended over a period of 3½ years, and embrace results from 700 pot cultures and 400 phosphorus determinations.

LITERATURE CITED

(1) BISHOP, W. H.
1894. Report of pot experiments with phosphates. Del. Agr. Exp. Sta. 6th Ann. Rpt., 1893, p. 193–202, illus.

(2) BROOKS, W. P.
1898. Natural phosphates compared with each other and with acid phosphate. *In* Mass. Hatch Agr. Exp. Sta. 10th Ann. Rpt., [1896/97], p. 14–19. Reviews work done by Goessmann.

(3) ———.
1901. The relative value of different phosphates. *In* Mass. Hatch Agr. Exp. Sta. 13th Ann. Rpt., [1899/1900], p. 101–105.

(4) DYER, B.
1884. Comparison of dissolved and undissolved phosphates. (Abstract.) *In* Jour. Chem. Soc. [London], v. 46, Abstracts, p. 774. 1884. Original article in Jour. Roy. Agr. Soc. England, s. 2, v. 20, p. 113–126. 1884. Not seen.

(5) FREAR, William.
1887. Agricultural experiment work for 1885 and 1886. II. Experiments with different kinds of phosphoric acid. Penn. State, Col. Rpt., 1886, p. 124–131.

(6) HARTWELL, B. L., and PEMBER, F. R.
1912. The effect of cow dung on the availability of rock phosphate. R. I. Agr. Exp. Sta. Bul. 151, p. 165–174, 1 pl.

(7) HESS, E. H.
1896. Experiments with soluble, reverted and insoluble phosphoric acid. Penn. Agr. Exp. Sta. Ann. Rpt., 1895, p. 157–210.

(8) HOPKINS, C. G., MOSIER, J. G., PETTIT, J. H., and FISHER, O. S.
1913. McDonough county soils. Ill. Agr. Exp. Sta. Soil Rpt. 7, 46 p., 7 fig., 2 col. maps.

(9) JOHNSON, S. W.
1890. Field experiments in 1889. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1889, p. 203–232.

(10) JORDAN, W. H.
1895. Field experiments with fertilizers. *In* Maine Agr. Exp. Sta. Rpt., 1894, p. 16–32.

(11) ———.
1913. Studies in plant nutrition. I. N. Y. Agr. Exp. Sta. Bul. 358, 30 p.

(12) KRÖBER, E.
1909. Über das Löslichwerden der Phosphorsäure aus wasserunlöslichen Verbindungen unter der Einwirkung von Bakterien und Hefen. *In* Jour. Landw., Bd. 57, Heft 1, p. 5–80.

(13) LUPTON, N. T.
1893. The effect of decomposing organic matter on natural phosphates. Ala. Agr. Exp. Sta. Bul. 48, 80 p.

(14) MCDOWELL, M. S.
1908. Is the phosphoric acid of floats made soluble by rotting manure? Penn. Agr. Exp. Sta. Ann. Rpt., 1907/08, p. 175–178.

(15) MERRILL, L. H.
1899. Box experiments with phosphoric acid from different sources. Maine Agr. Exp. Sta. 14th Ann. Rpt., 1898, p. 64–74, 7 pl.

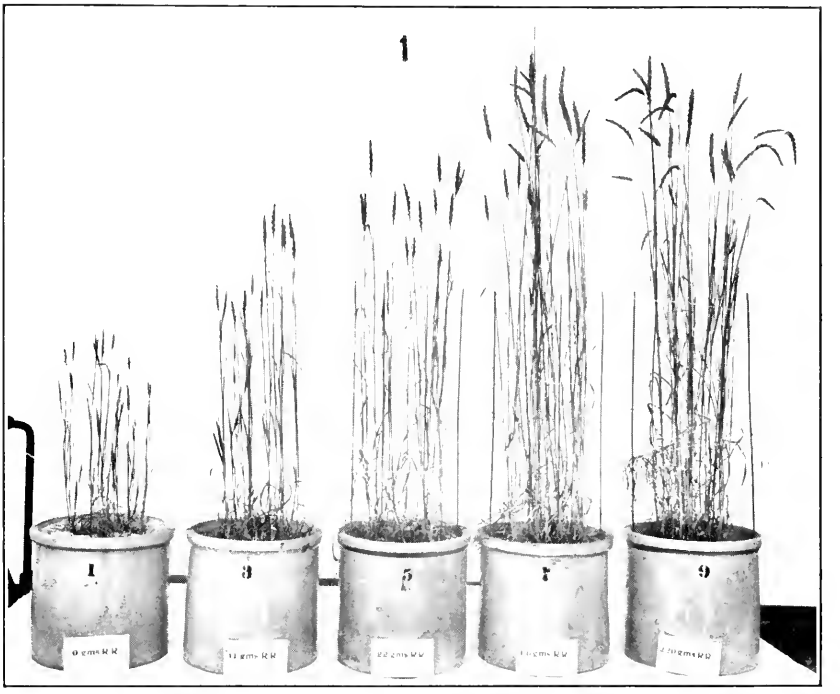
(16) NEWMAN, J. S.
1889. Cotton—experiments with fertilizers. *In* Ala. Agr. Exp. Sta. Bul. 5, n. s., p. 3–13.

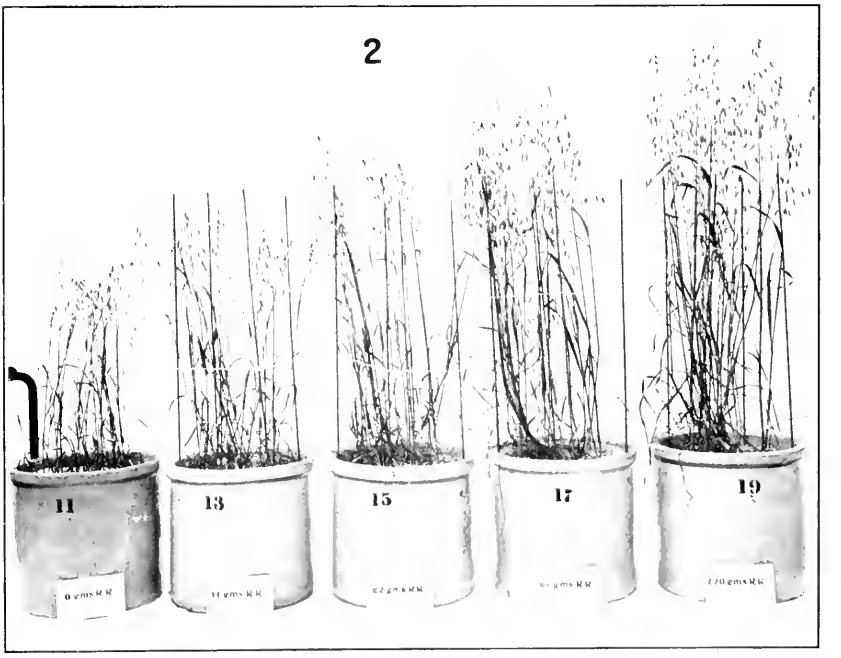
(17) NEWMAN, J. S., and CLAYTON, James.
1891. Experiments with cotton—1890. Ala. Agr. Exp. Sta. Bul. 22, n. s., 29 p.
(18) PATTERSON, H. J.
1907. Fertilizer experiments with different sources of phosphoric acid. Md. Agr. Exp. Sta. Bul. 114, p. 113–144.
(19) PFEIFFER, Th., and THURMAN, H.
1896. Über das Verhalten einiger Phosphate bei der Kompostierung. *In* Landw. Vers. Stat., Bd. 47, Heft 4/5, p. 343–356.
(20) PRIANICHNIKOW, D. N.
1899. Étude sur la valeur relative des phosphates minéraux. *In* Ann. Agron., t. 25, no. 4, p. 177–187.
(21) RUSSELL, H. L.
1913. Report of the director. Availability of phosphate to various crops. Wis. Agr. Exp. Sta. Bul. 240, p. 22–23, fig. 11.
(22) SCHLOESSING, T., jr.
1902. A study of the phosphorus nutrition of plants. (Abstract.) *In* Exp. Sta. Rec., v. 14, no. 3, p. 233. 1902. Original article in Compt. Rend. Acad. Sci. [Paris], t. 134, no. 1, p. 53–55. 1902. Not seen.
(23) SCHUTT, F. T.
1897. Report of the chemist. The fermenting of manure with finely ground mineral phosphate. *In* Canada Exp. Farms Rpts., 1896, p. 196.
(24) [THORNE, C. E.]
1910. Plans and summary tables of the experiments at the central farm, Wooster, on the maintenance of soil fertility. Ohio Agr. Exp. Sta. Circ. 104, 20 p.
(25) ———
1913. Farm manures. 242 p., illus. New York, London.
(26) TOTTINGHAM, W., E., HOFFMAN, C.
1913. Nature of the changes in the solubility and availability of phosphorus in fermenting mixtures. Wis. Agr. Exp. Sta. Research Bul. 29, p. 273–321, 3 fig.
(27) TRUOG, E.
1912. Factors influencing the availability of rock phosphate. Wis. Agr. Exp. Sta. Research Bul. 20, p. 17–51, 6 fig.
(28) Verband landwirtschaftlicher Versuchs-Stationen im Deutschen Reiche.
1907. Vorläufige Mitteilung der Beschlüsse der XXIV. Hauptversammlung des Verbandes zu Dresden am 14. September 1907. *In* Landw. Vers. Stat., Bd. 67, Heft 5/6, p. 321–329.
(29) VOELCKER, A.
1880. Comparative value of soluble and insoluble phosphates. (Abstract.) *In* Jour. Chem. Soc. [London], v. 40, Abstracts, p. 640–641. 1881. Original article in Jour. Roy. Soc. Agr. Soc. England, s. 2, v. 16, p. 152–159. 1880. Not seen.
(30) WHEELER, H. J., and ADAMS, G. E.
1900. The needs and treatment of the Warwick Plain and other sandy soils of Rhode Island. R. I. Agr. Exp. Sta. Bul. 68, p. 159–174.
(31) ——— ———
1906. A comparison of nine different phosphates upon limed and unlimed land with several varieties of plants. R. I. Agr. Exp. Sta. Bul. 114, p. 117–137.
(32) ——— ———
1907. Continued test of nine different phosphates upon limed and unlimed land with several varieties of plants. R. I. Agr. Exp. Sta. Bul. 118, p. 55–86.

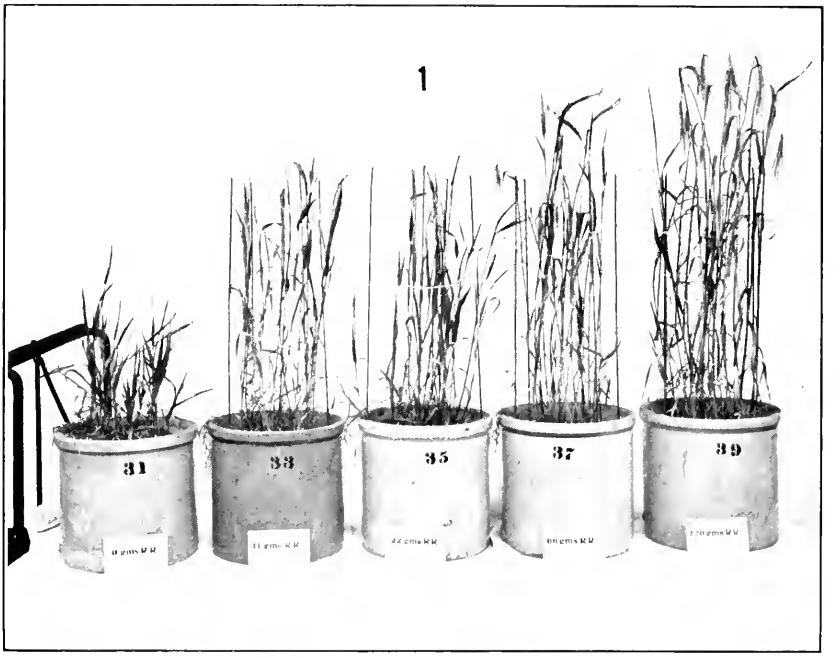
PLATE LXXIII

Effect of varying quantities of Tennessee brown rock phosphates on plant growth:

Fig. 1.—Spring wheat. (Table II, Series 1A.)
Fig. 2.—Sixty-Day oats. (Table II, series 1B.)


1
1
3
5
7
9


2
11
13
15
17
19


1
31
33
35
37
39

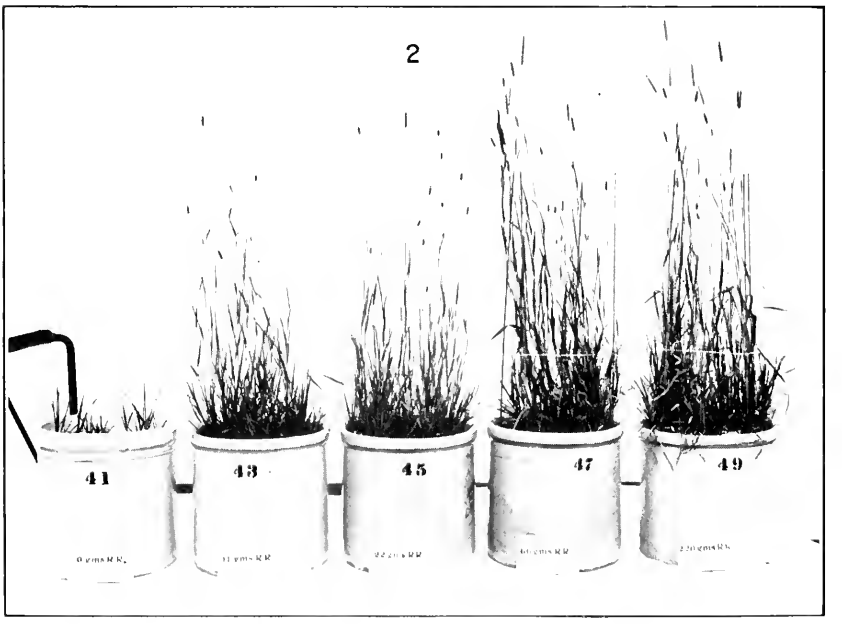

2
41
43
45
47
49

PLATE LXXIV

Effect of varying quantities of Tennessee brown rock phosphate on plant growth:

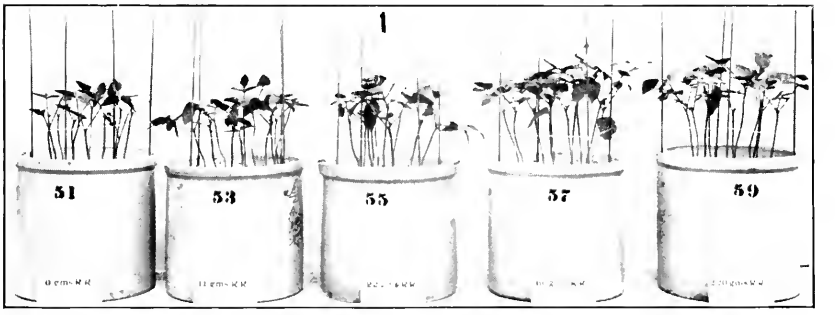
Fig. 1.—Barley. (Table VI.)
Fig. 2.—Timothy. (Table III, series 1E.)

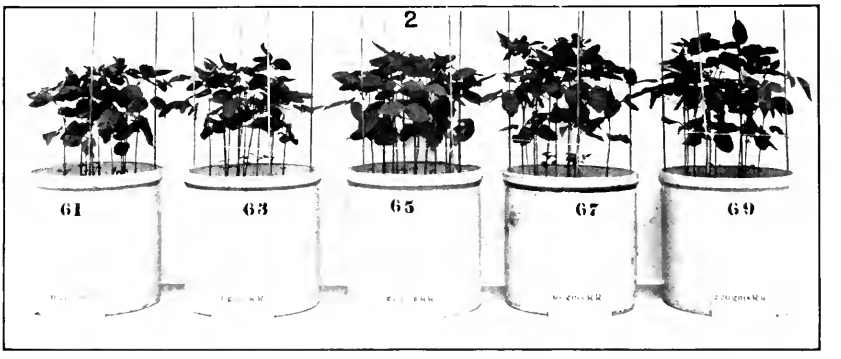
37770°—16——4

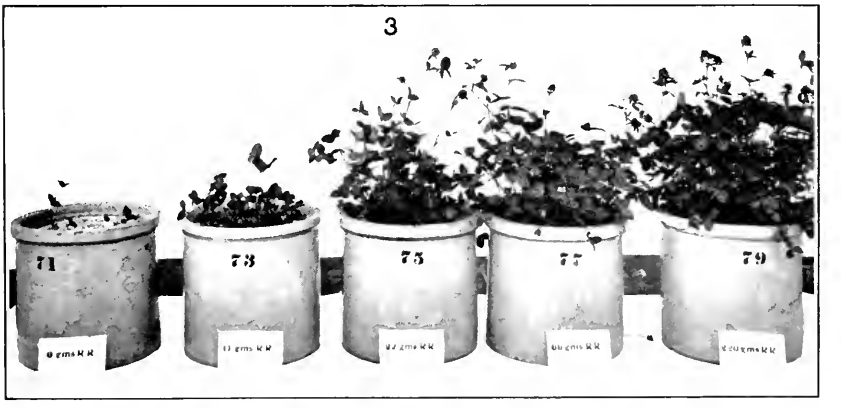
PLATE LXXV

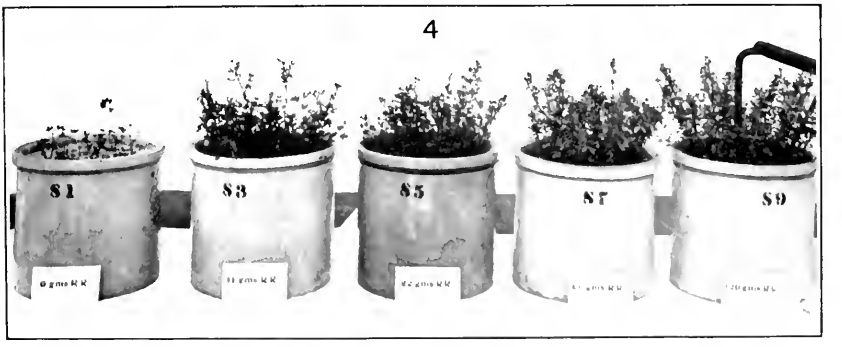
Effect of varying quantities of Tennessee brown rock phosphate on plant growth;

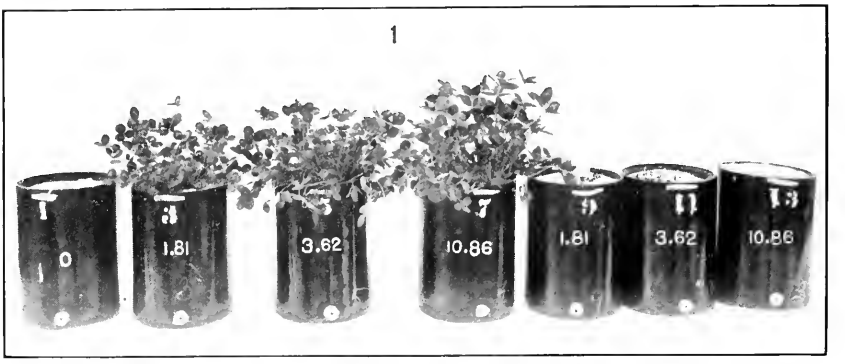
Fig. 1.—Cowpeas. (Table IV, series 1F.)
Fig. 2.—Soybeans. (Table IV, series 1G.) Photographed just before cutting.
Fig. 3.—Red clover. (Table III, series 1H.)
Fig. 4.—Alfalfa. (Table V.) Photographed before first cutting.

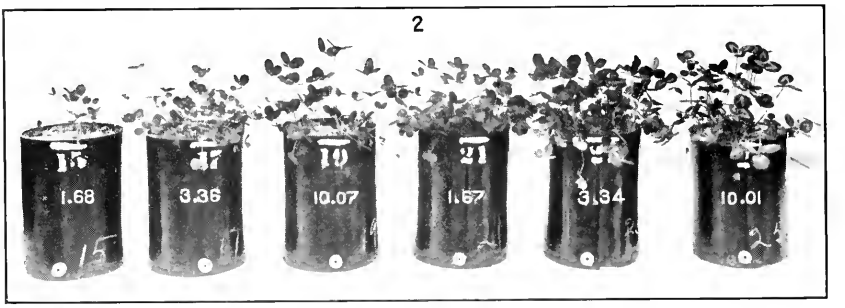

1
51
53
55
57
59


2
61
63
65
67
69


3
71
73
75
77
79


4
81
83
85
87
89


1
0
1.81
3.62
10.86
1.81
3.62
10.86


2
1.68
3.36
10.07
1.67
3.34
10.01

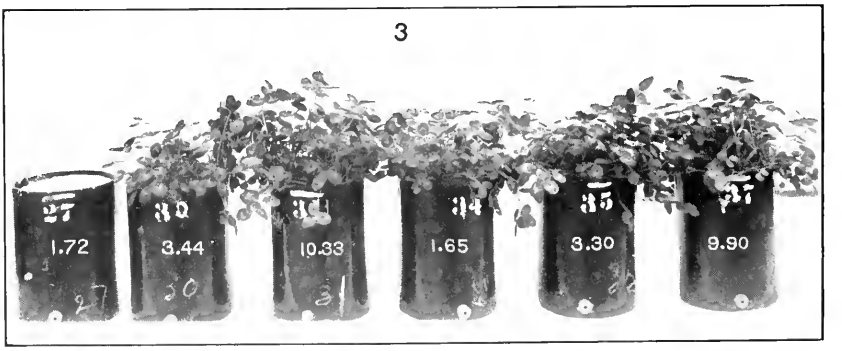
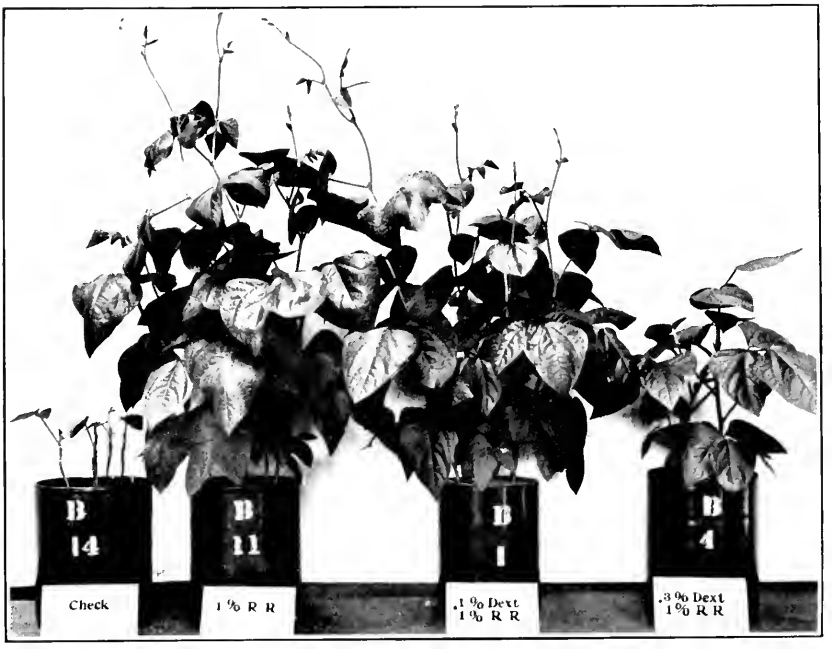

3
1.72
3.44
10.33
1.65
3.30
9.90

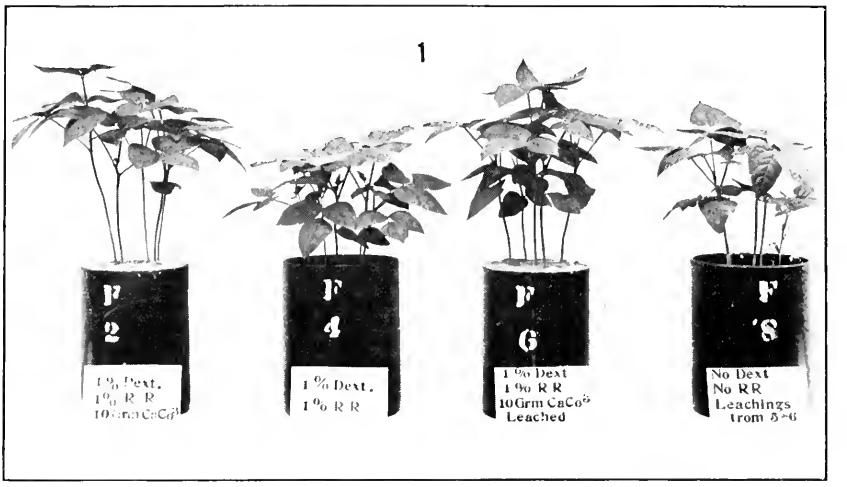
PLATE LXXVI

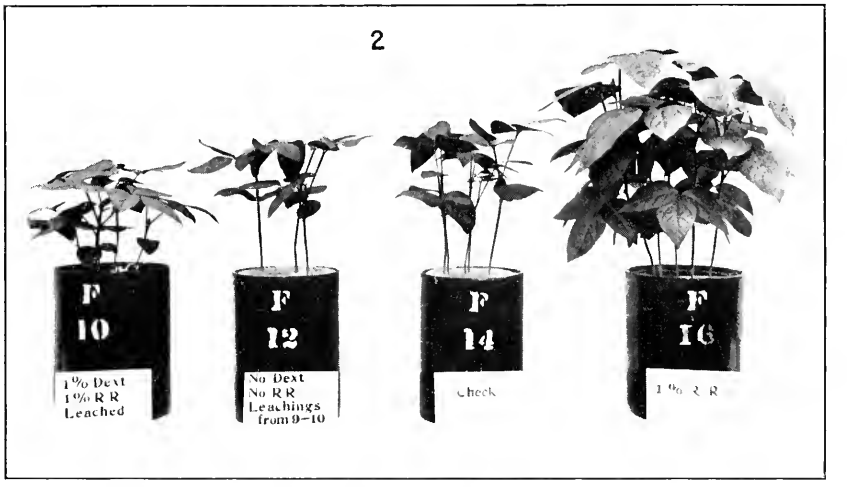
Effect of different kinds of mineral phosphate applied in different quantities for red clover. (Table VII.) Photograped just before first cutting.

PLATE LXXVII

Cowpeas, showing the comparative effect of Tennessee brown rock phosphate alone and in combination with dextrose. (Table XII.)


B
14
Check
B
11
1% R R
B
1
.1% Dext
1% R R
B
4
.3% Dext
1% R R


1
F 2
1% Dext. 1% R R 10 Grm CaCo3
F 4
1% Dext. 1% R R
F 6
1% Dext 1% R R 10 Grm CaCo3 Leached
F 8
No Dext No R R Leachings from 5-6


2
F 10
1% Dext 1% R R Leached
F 12
No Dext No R R Leachings from 9-10
F 14
Check
F 16
1% R R

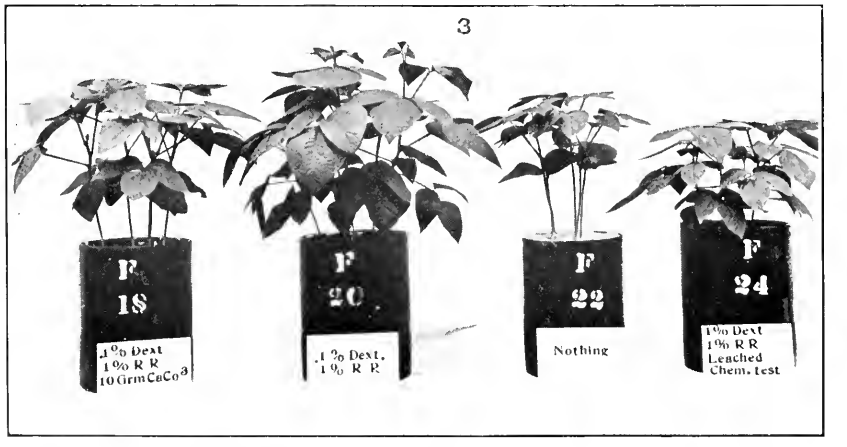

3
F 18
1% Dext 1% R R 10 Grm CaCo3
F 20
1% Dext. 1% R R
F 22
Nothing
F 24
1% Dext 1% R R Leached Chem. test

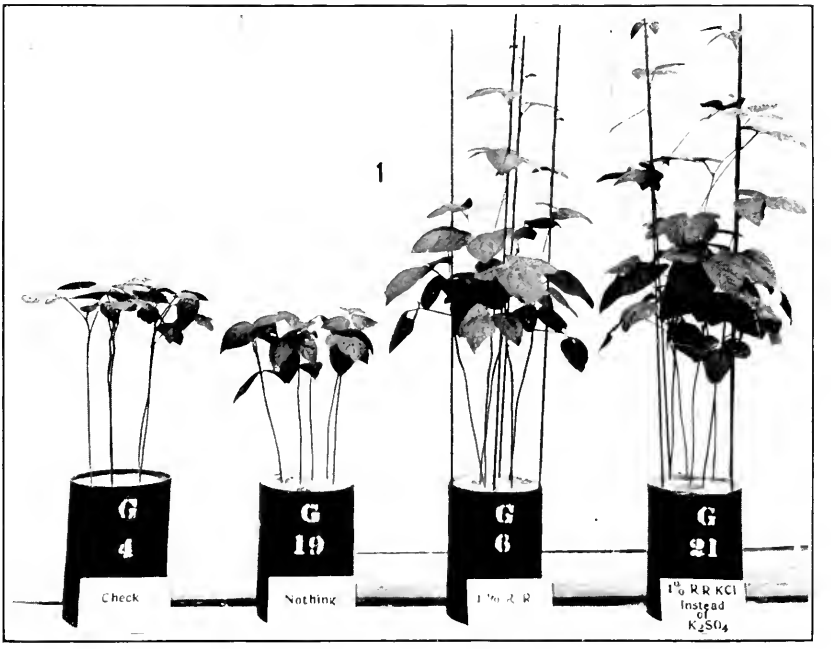
PLATE LXXVIII

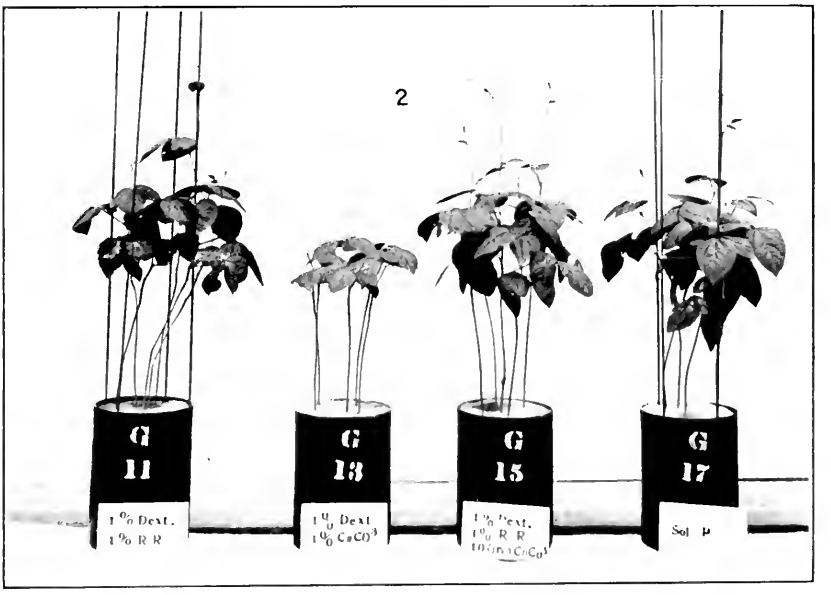
Cowpeas, showing the comparison of their growth when treated with Tennessee brown rock phosphate, phosphate and dextrose, and phosphate, dextrose, and calcium carbonate. (Table XIV.) Photographed just before harvesting.

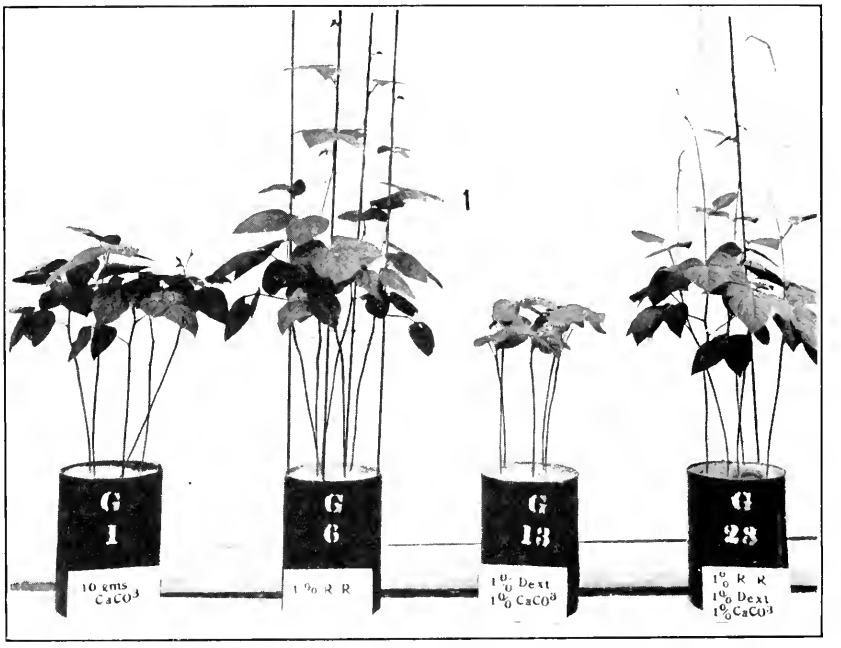
PLATE LXXIX

Effect of different substances on the growth of cowpeas:

Fig. 1.—Growth after the addition of varying quantities of raw rock. (Table XV.)
Fig. 2.—Growth after the addition of dextrose and soluble phosphate. (Table XV.)


1
G 4
Check
G 19
Nothing
G 6
G 21
1% R R KCl Instead of K_2SO_4


2
G 11
1% Dext. 1% R R
G 13
1% Dext. 1% $CaCO^3$
G 15
G 17
Sol P


1
G 1
10 gms CaCO3
G 6
1% R R
G 13
1% Dext 1% CaCO3
G 28
1% R R 1% Dext 1% CaCO3

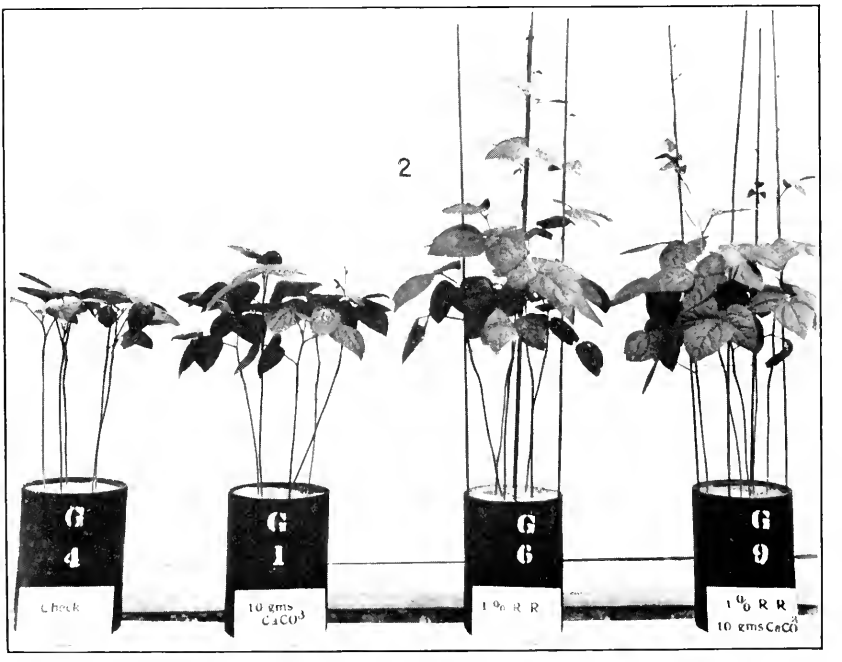

2
G 4
Check
G 1
10 gms CaCO3
G 6
1% R R
G 9
1% R R 10 gms CaCO3

PLATE LXXX

Effect of various substances and combinations on the growth of cowpeas:

Fig. 1.—Effect of adding lime, phosphate rock, dextrose and lime, and phosphate rock, dextrose, and lime to the soil. (Table XV.)

Fig. 2.—Effect of adding nothing, lime, phosphate rock, and phosphate rock and lime to the soil. (Table XV.)

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

20 CENTS PER COPY
SUBSCRIPTION PRICE, $3 PER YEAR