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THE EFFECTS OF KEROSENE AND OTHER PETROLEUM OILS ON THE VIABILITY AND GROWTH OF ZEA MAIS

BY

JOHN HAMILTON WHITTEN
A.B., University of Illinois, 1911
A.M., University of Illinois, 1912

THESIS

Submitted in Partial Fulfilment of the Requirements for the

Degree of

DOCTOR OF PHILOSOPHY

IN BOTANY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

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ARTICLE V.—*The Effects of Kerosene and other Petroleum Oils on the Viability and Growth of Zea mais.* BY JOHN H. WHITTEN, PH.D.

I. INTRODUCTION

It has long been a custom among farmers to pour kerosene over seed corn just previous to planting in order to protect it from being injured or destroyed by squirrels, crows, or other pests. Until recently few or no careful observations had been made to determine whether the treatment accomplished the purpose for which it was used or if the effects on germination and subsequent growth were favorable or otherwise.

Lummis ('03), from a few preliminary experiments in field and laboratory, reported a decided reduction in the percentage of germination and a very conspicuous injury to growth. Forbes ('08), seeking a repellent particularly against the corn root-aphis, gave the kerosene treatment a much more extensive trial. He found it very effective in repelling the corn-field ant and consequently in controlling the aphis, but he reports the frequent occurrence of "perplexing discrepancies" in respect to the effects of the treatment on germination and growth. His data show that in some instances corn was very markedly injured, while in others a similar treatment produced no detrimental effect; and that a brief treatment—thirty minutes' immersion—was more injurious than one of twenty days. Having no explanation for such results and not desiring to enter into the analysis of a complex problem of plant physiology, Professor Forbes abandoned the use of kerosene, but he was free to admit that the scope of his investigations was too limited to warrant general conclusions.

Duggar and McCool ('09), issued a circular of information intended especially for the constituency of the Cornell University Experiment Station. In this report the authors made no pretension to original investigation on the use of kerosene as a deterrent. They laid particular stress on Professor Forbes's work and made their recommendations accordingly.

Previous to the publication of the bulletin above cited there had been some work done on the same subject under the direction of Dr. Hottes in the laboratory of plant physiology at the University of

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Illinois. This work was not completed, but from the results obtained and from the report published by Professor Forbes, Professor Hottes was led to believe that the physiological aspects of the subject were of sufficient interest to warrant a more thorough investigation, and it was at his suggestion and under his direction that the work herein described was undertaken. It is with pleasure that I acknowledge the helpful advice and constructive criticism offered by Professor Hottes during the progress of the work.

II. Methods

In a series of initial experiments an attempt was made to learn something of the conditions which affect the germination of corn after immersion in kerosene, and thus to discover, if possible, the causes of the "perplexing discrepancies" referred to by Professor Forbes. Very early in this work it became apparent that the highest per cent. of germination could be secured only when the oil on the surface of or within the grain had been reduced to the minimum. The effect of the oil became especially marked when the corn was placed under conditions for germination with more than the usual amount of water present. These preliminary experiments further showed that the presence of water in the grain at the time of immersion in kerosene was responsible in no small degree for the wide variation in the per cent. of germination.

1. Elimination of the Oil

The superficial oil was removed from the grains by careful wiping with soft, absorptive towels immediately after removing them from the kerosene. The grains were then imbedded in desiccated powdered clay or plaster of Paris to remove as much of the remaining oil as possible. The same result was accomplished, and much more simply, by placing the corn on dry filter-paper exposed to the air to allow the kerosene to volatilize. In a few instances when the corn had been immersed for long periods of time the gummy coating formed on the outside of the grain was dissolved off by washing vigorously for a few minutes in chloroform. Acetone was also tried for the same purpose, but it proved to be injurious to control grains and its use was discontinued. Since exposure to dry air at room temperature, 23 to 28 degrees C., gave results in no wise inferior to the other methods used for eliminating kerosene, it was used almost exclusively in all cases where the elimination of the oil was called for.

2. SOAKING IN WATER

Grains which had been immersed in kerosene were soaked in water for from one to forty-eight hours and then placed, together with a check, under the usual conditions for germination. It was found that soaking in running water for periods of from one to eight hours was not injurious and in a few instances seemed slightly beneficial. When soaked for longer periods a decided reduction in the per cent. of germination was manifested. Abnormalities in the later growth of the seedlings from grains immersed in kerosene and subsequently soaked in water for the shorter periods were more frequent than from unsoaked grains. On the whole it seemed clear that the smaller the amount of water present in the grain at the time of planting and consequently the more slowly the growth processes were initiated the less serious were the effects of the kerosene.

3. MOISTURE CONTENT OF THE SOIL

The moisture content of the soil was varied from the minimum amount necessary for germination to complete saturation. The results were so diverse that it was decided to make three sets of tests, identical in every way except the water content of the soil. The first contained approximately the minimum amount in which germination would take place readily; the second, the maximum amount in which germination and normal growth of control grains could be secured; and the third, an amount which represented an approximate mean to the other two. Different soils vary so widely in their power to hold water that the percentages of saturation used to secure the conditions indicated above must necessarily be determined by experiment and can only be made to apply to the particular soil used.

4. CULTURE MEDIA

The grains were germinated in the ordinary germinating pans, in yellow clay, sand, sawdust, black loam, and in various mixtures of sand and sawdust and of sand and black loam. Of these the sawdust was the only one which showed any harmful effects. The use of the germinating pan was early discontinued because of the necessity of making observations on the growth for some time following germination. A mixture of black loam and sand yielded as good results as any other, and this was selected because of its adaptability for varying and maintaining the moisture content.

5. PREPARATION OF THE SOIL

A quantity of potting soil, a very rich black loam, was obtained from the university greenhouse. It had been prepared by piling up turf and allowing the vegetable matter to decompose. After drying to a sufficient degree to render it suitable for handling, the soil was screened through a sieve with one-twelfth inch mesh and mixed with sand, similarly screened, in the proportion of two parts of loam to one part of sand. These two constituents were worked into a perfectly homogeneous mass, and its capacity for holding water was determined by the following method:

A cu. dm. of the prepared soil, which weighed 1 kilo, was dried to constant weight in an oven at 100 degrees C. After the constant weight had been secured, the dry soil was placed on filter-paper in a funnel and water was added in small quantities at frequent intervals. When the soil was uniformly moist and no longer increased in weight after the surplus water had drained off, it was again weighed, and thus the quantity of water necessary to saturate was determined. Soil in this condition was considered to be 100% saturated. The different percentages of saturation used were computed on the basis of the total amount of water in the saturated soil.

The following table shows the records of a few typical tests made to determine the capacity of the soil for holding water.

Wt. of norm. soil	Wt. of dry soil	Wt. of H_2O in norm. soil	Wt. of sat. soil	Wt. of H_2O in sat. soil	% of sat. of norm. soil
1020	943	77	1302	359	21
1000	928	72	1253	325	22
1000	924	76	1262	338	22
938	851	87	1201	350	24.8

By exercising a little care, the moisture content of the soil could be adjusted at the beginning of each test so that one cu. dm. of soil would weigh 1 kilo. This was selected as the standard weight and somewhat arbitrarily designated as "normal soil."

The difference between the weight of the normal soil and the weight of the dry soil is the weight of the water in the normal soil.

The difference in the weights of the dry and the saturated soils is the weight of water necessary to produce 100% saturation. Knowing these facts it becomes a simple matter to add water to the normal soil in quantities required to make it any desired per cent. of saturation.

The same soil was used repeatedly, but it was carefully screened and brought to "normal weight" before using.

When a 30% saturation or less was desired, it was possible to mix thoroughly the soil and the required amount of water without puddling. Soil containing higher percentages of saturation could not be prepared in this manner. The most convenient and efficient way of securing a rapid and an equal distribution of the water in 50 and 75% saturations was to place a thin layer of soil in the pan and spray it with the proportionate amount of water, then add another layer and spray again. This process was continued until the desired amount of soil was in the pan and the proper amount of water added to give the required moisture content. In a soil thus treated and protected to avoid evaporation, the moisture was found uniformly distributed in a short time.

6. PLANTING

The grains were planted in rectangular pans 35 cm. by 20 cm. and 7 cm. deep. These pans were provided with closely fitting covers in which were openings for aëration.

Each pan was furnished with 4 cu. dm. of prepared soil, in which the grains were planted and then uniformly covered with an additional cu. dm. of soil. The grains were placed one-half inch apart in rows one inch apart. As an aid in securing an equal distribution and thus in observing the behavior of the individual grains, a wire netting with one-half inch mesh was placed on the surface of the soil and the grains were inserted through it at regular intervals. They were thrust into the soil in an upright position until the butts were flush with the surface, and were then covered with the final cu. dm. of soil. To prevent the grains from being pushed out of the loose soil of the low moisture cultures by the growth of the root, the soil was slightly compressed by placing a glass plate on the surface and applying a slight pressure with the hand both before and after the addition of the soil used for covering.

The gross weight of the pan and contents was then taken. The pans used were provided with a shoulder just beneath the cover which served to support a wire netting on which was kept moist blotting paper. In this way the loss of water was greatly reduced, amounting to only 2 or 3 c.c. in twenty-four hours. The original weight was kept constant by the addition of water each morning by means of an atomizer.

The pans were kept covered during the period of germination and until the seedlings were well through the soil. The critical period of the test then being passed, the covers were removed to admit light, and no further effort was made to keep the moisture content constant. Water was added in sufficient quantities to keep the corn in good growing condition.

The method used for securing and maintaining the moisture content of the soil is not absolutely accurate. The limits of error, however, were certainly within 2%, which was sufficiently accurate for the purpose intended.

7. TEMPERATURE

During the periods of germination and initial growth the cultures were kept in a basement room where the temperature varied between 23 and 28 degrees C. After the seedlings were well above the surface of the soil the covers of the pans were removed and the cultures taken to the greenhouse, where the corn was allowed to grow until it was from five to six inches tall. It was then taken up and all abnormal seedlings were carefully examined.

III. Experimentation and Discussion

1. EFFECTS OF THE PERIOD OF IMMERSION AND THE MOISTURE CONTENT OF THE SOIL

As already indicated, the water content of the soil in which the grains, after immersion in kerosene, are planted has a very marked effect on germination. The data that follow in tables 1 to 4 inclusive show the per cent. of germination and normal growth as affected by the period of immersion in kerosene and the water content of the soil.

The kerosene used was a product of the Standard Oil Company, on the market under the trade name "Perfection."

In all tests recorded in the tables, the grains were carefully selected before immersing them in the kerosene, but no selection was made after removing them from the oil.

The time indicated under the heading "kerosene treatment" gives the period during which the grains were immersed in the oil. In the column "after treatment" is indicated the treatment of the grains after removal from the kerosene and before planting. In every case where it is not specifically stated to the contrary, the grains were superficially dried with a towel immediately after removing them from the oil. When this was not done it is indicated in the table by the word "none." The grains in these instances were planted directly from the kerosene.

A grain was considered germinated if it showed a decided growth (1 inch) of radicle or coleoptile.

Under the heading "No. injured" are recorded the number of seedlings showing injuries of one form or another resulting from the treatment.

The variety of corn used in the experiments of Series A, Tables 1, 2, and 3, was Boone County White; Table 4, Golden Eagle. In all other experiments Champion White Pearl was used. The first two are dent varieties. The Champion White Pearl has a large, hard, smooth grain. Extended comparisons between it and the dent varieties showed but slight differences in respect to the effects of the kerosene.

Series A

Tables 1 to 4 inclusive. Germination and growth as affected by the period of immersion in kerosene and the moisture content of soil.

Table 1. Boone County White Corn in a 30% Saturated Soil

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	Per ct. norm. growth
1	50	dipped	3 da. air	50	0	100	100
	50	1 min.	,,	50	1	100	98
	50	5 min.	,,	50	1	100	98
Check	25			25	1	100	96
2	50	10 min.	3 da. air	50	0	100	100
	50	15 min.	,,	50	0	100	100
	50	30 min.	,,	50	3	100	94
Check	25			25	1	100	96
3	50	1 hr.	3 da. air	50	1	100	98
	50	2 hr.	,,	50	0	100	100
	50	3 hr.	,,	50	1	100	98
	50	6 hr.	,,	50	2	100	96
	50	8 hr.	,,	50	0	100	100
	50	14 hr.	,,	50	0	100	100
Check	50			50	0	100	100
4	50	1 da.	3 da. air	50	0	100	100
	50	3 da.	,,	50	2	100	96
	50	6 da.	,,	50	0	100	100
Check	25			25	0	100	100
4a	100	1 da.	5 da. air	100	2	100	98
	100	3 da.	,,	100	1	100	99
	100	6 da.	,,	100	2	100	98
Check	25			25	0	100	100
5	100	10 da.	5 da. air	92	5	92	87
Check	25			25	0	100	100
6	100	25 da.	5 da. air	85	3	85	82
Check	25			25	0	100	100
7	100	30 da.	5 da. air	86	10	86	76
Check	25			24	0	96	96
8	100	50 da.	5 da. air	76	3	76	73
Check	25			25	0	100	100
9	100	120 da.	5 da. air	72	5	72	67
Check	25			25	0	100	100
10	100	158 da.	5 da. air	64	0	64	64
Check	25			25	1	100	96
11	100	190 da.	5 da. air	57	0	57	57
Check	25			25	0	100	100
12	100	215 da.	5 da. air	60	2	60	58
Check	25			25	0	100	100
13	100	1 yr.	5 da. air	66	0	66	66
Check	25			25	1	100	96
14	100	2 yrs.	5 da. air	56	0	56	56

Series A—*Continued*

Table 2. Boone County White Corn in a 50% Saturated Soil

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	Per ct. norm. growth
1	50	dipped	3 da. air	41	1	82	80
	50	1 min.	,,	44	1	88	86
	50	5 min.	,,	45	1	90	88
Check	25			25	0	100	100
2	50	10 min.	3 da. air	40	0	80	80
	50	15 min.	,,	44	1	88	86
	50	30 min.	,,	42	1	84	82
Check	25			24	1	96	92
3	50	1 hr.	3 da. air	43	2	86	82
	50	2 hr.	,,	40	2	80	76
	50	3 hr.	,,	38	2	76	72
	50	6 hr.	,,	36	1	72	70
	50	8 hr.	,,	38	1	76	74
Check	50			49	1	98	96
4	50	1 da.	3 da. air	36	1	72	70
	50	3 da.	,,	37	2	74	70
	50	6 da.	,,	33	1	66	64
Check	25			25	1	100	96
5	100	10 da.	5 da. air	60	1	60	58
Check	25			25	0	100	100
6	100	25 da.	5 da. air	61	8	61	53
Check	25			25	0	100	100
7	100	30 da.	5 da. air	62	3	62	59
Check	25			24	0	96	96
8	100	50 da.	5 da. air	60	3	60	57
Check	25			25	0	100	100
9	100	120 da.	5 da. air	56	0	56	56
Check	25			24	1	96	92
10	100	158 da.	5 da. air	56	1	56	55
Check	25			25	0	100	100
11	100	190 da.	5 da. air	53	0	53	53
Check	25			100	0	100	100
12	100	215 da.	5 da. air	47	2	47	45
Check	25			23	0	92	92
13	100	1 yr.	5 da. air	48	0	48	48
Check	25			25	0	100	100
14	100	2 yrs.	5 da. air	40	0	40	40
Check	25			25	0	100	100

Series A—*Continued*

Table 3. Boone County White Corn in a 75% Saturated Soil

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	Per ct. norm. growth
1	50	dipped	3 da. air	27	0	54	54
	50	1 min.	”	30	1	60	58
	50	5 min.	”	26	1	52	50
Check	25			24	1	96	92
2	50	10 min.	3 da. air	28	1	56	54
	50	15 min.	”	33	1	66	64
	50	30 min.	”	30	0	60	60
Check	25			23	2	92	84
3	50	1 hr.	3 da. air	31	0	62	62
	50	2 hr.	”	28	2	56	52
	50	3 hr.	”	25	1	50	48
	50	6 hr.	”	22	1	44	42
	50	8 hr.	”	23	0	46	46
	50	14 hr.	”	27	5	54	44
Check	50			45	1	90	88
4	50	1 da.	3 da. air	25	2	50	46
	50	3 da.	”	30	6	60	48
	50	6 da.	”	22	2	44	40
Check	25			23	1	96	88
5	100	10 da.	5 da. air	42	3	42	39
Check	25			24	0	96	96
6	100	25 da.	5 da. air	40	4	40	36
Check	25			25	0	100	100
7	100	30 da.	5 da. air	41	2	41	39
Check	25			23	0	92	92
8	100	50 da.	5 da. air	35	1	35	34
Check	25			25	1	100	96
9	100	120 da.	5 da. air	28	2	28	26
Check	25			25	0	100	100
10	100	158 da.	5 da. air	22	2	22	20
Check	25			24	0	96	96
11	100	190 da.	5 da. air	25	4	25	21
Check	25			23	0	92	92
12	100	215 da.	5 da. air	26	5	26	21
Check	25			25	2	100	92
13	100	1 yr.	5 da. air	12	4	12	8
Check	25			24	0	96	96
14	100	2 yr.	5 da. air	8	2	8	6
Check	25			25	1	100	96

2. LONG PERIODS OF IMMERSION

Reference has already been made to the work started on this problem in the laboratory of plant physiology at the University of Illinois previous to the publication of the report by Professor Forbes ('08). This work was abandoned before any definite results had been obtained, but the corn immersed in kerosene at that time (February 6, 1906) was set aside and kept in the storeroom of the laboratory in a loosely covered fruit-jar until the present work was begun. The oil had become yellow and was of the consistency of thin syrup. There was about a pint of this corn—an amount far too small to permit any elaborate tests, but sufficient to demonstrate conclusively that under optimum conditions a considerable portion of it was capable of germination and perfectly normal growth.

The majority of the trials recorded in Table 4 were made when the preliminary experiments, already referred to, were in progress. Of these trials, No. 3 yielded the highest per cent. of germination and was in every way the most satisfactory of any which had been made up to that time. After the treatment indicated in the table, the grains used in this trial were placed on filter-paper in a germinating pan with barely enough moisture present to initiate the growth processes. As soon as a definite growth of root and coleoptile appeared the grains were transferred to soil in which the moisture was somewhat higher but which did not exceed 30% saturation. This method was followed in all subsequent trials made with this corn. The seedlings recorded in the column under *per cent. of normal growth* were just as vigorous and had just as good color as the check seedlings which were grown from corn less than one year old. Plate XVI is a picture of two stalks of the corn grown from grains immersed in kerosene for eight years (Trial 9, Table 4).

A number of attempts were made to germinate grains of this corn in 50 and 75% saturated soil but all were complete failures.

SERIES A—*Concluded*

TABLE 4.

Golden Eagle Corn, immersed in kerosene February 6, 1906. Trial 1 was made July 11, 1911. The others followed as the time of the kerosene treatment indicates. Soil 30% saturated.

Trial	No. grains	Kerosene treatment	After-treatment	Per ct. germ.	% Norm. growth
1	50	5 yrs, 5 mos., 5 da.	48 hrs. clay, 12 hrs. air	40	40
2	50	"	48 hrs. plaster of Paris, 12 hrs. air	44	44
3	100	5 yrs., 5 mos., 22 da.	7 da. plaster of Paris, 1 da. air, 8 hrs. running water	64	55
4	35	"	60 da. plaster of Paris	65	56
5	35	"	None	32	32
6	25	5 yrs., 6 mos., 8 da.	60 da. plaster of Paris	58	50
7	25	"	60 da. air	44	36
8	20	6 yrs., 2 mos.	10 da. air	50	40
9	20	8 yrs.	5 min. chloroform, 5 da. air	20	15

3. TYPES OF ABNORMALITIES

In soils of 50 and 75% saturation, abnormally swollen grains occurred frequently. A watery fluid collected inside the membranes in considerable quantities. By slight pressure several drops could be secured from a single grain. Microscopical examination of the extracted liquid showed the presence of both perfect and corroded starch grains. Occasionally in the 75% saturated soil this liquid seemed to undergo fermentation. The pericarp, in these instances, was ruptured and the accumulated liquid made its way to the surface of the soil where it spread out and, drying, formed a hard white crust. Examination of this crust under the microscope showed corroded starch grains and a large number of bacteria. The bacteria were of uniform shape and size and apparently belonged to a single species. Swellings as above described occurred among the grains which had failed to germinate and among those which were growing normally. They were also occasionally found among the normal grains used as a check, but much less frequently than among the treated grains. From the observations made, there was nothing to indicate that the swollen condition had any bearing on the germination or growth of the corn.

When punctured by a pin-prick the liquid inside the grain oozed out and no further accumulation of it occurred. This showed that it probably was due to the high osmotic pressure inside the intact membranes and to an abundance of available water in the surrounding soil. No swollen grains appeared in cultures in which the moisture content of the soil was but 30% of saturation or less.

One of the most noticeable injuries, though by no means the most frequent, was a curled and twisted condition of the leaves due to their inability to unfold normally in the process of growth. An examination of the tips of these leaves showed, in the majority of cases, that they were dead and that they adhered to each other on that account. It was possible to produce typical cases of the injury on control seedlings by touching the tip of the growing shoot immediately after it appeared through the coleoptile with an injurious reagent. Of the reagents used for this purpose sulphuric acid was the most certain to cause the abnormal growth. Kerosene applied in the same manner produced the injury, but it was by no means as effective as the sulphuric acid. The injury appeared occasionally among control seedlings, but there can be no doubt that the unusual frequency of the deformity in treated grains was due to the effects of the kerosene.

Another abnormality attributable to the kerosene was a much enlarged and thickened coleoptile which the growing plumule occasionally failed to rupture. Whenever this unusual development appeared it was observed that the plumule had not grown nearly as far in the coleoptile as it ordinarily does. In some instances the plumule failed to develop, leaving the coleoptile entirely empty. This seemed to indicate that the coleoptile is less sensitive to the kerosene than the enclosed structures are, and that the enlargement is correlated with the failure of the plumule to develop.

The most frequent injury was the death of the shoot. This occurred many times in grains from which the root grew normally. Very rarely in these experiments did the shoot grow when the root had been killed.

The injuries mentioned above were not as distinct from each other as the descriptions might seem to indicate. As a matter of fact there was an imperceptible gradation from one to another. The deformed leaves seem to represent the first visible injurious effects of the kerosene treatment. Increasing ill effects, due to an increase in the period of immersion, could be followed through a gradually decreasing vitality, to death. The action of the kerosene in producing injuries, and other evidences to be presented later, indicate that kerosene is not a violent poison to the growing corn-seedling.

4. DRY MEMBRANES

It is evident that the kerosene did not act uniformly on the grains of corn which were subjected to the treatment. Some were killed, some injured, while others showed no injurious effects whatsoever. These conditions prevailed regardless of the period of immersion. No length of treatment was found which directly killed or even injured all the grains. This fact becomes significant when it is noted that the kerosene treatment was varied from a mere dipping to continuous immersion for a period of eight years.

Rather early in my work it was suspected that the oil penetrated the membranes of some grains more readily than those of others. Some embryos had an oil-soaked appearance after the kerosene treatment while others seemed free from the oil.

To obtain further evidence of the permeability of the coats to the oil, 200 grains of Champion White Pearl were placed in kerosene colored with Sudan III. After 50 days' immersion the corn was removed and superficially dried with a towel. One hundred of these grains, taken at random, were cut transversely through the middle of the embryos and carefully examined for the presence of colored oil. Seventy-eight showed no trace of oil or color in the embryos; five were slightly stained; the remaining seventeen were deeply stained and showed the presence of oil in considerable quantities. In no case was there any evidence of oil in the endosperm. The remaining 100 grains treated with Sudan III kerosene, as above indicated, were left exposed to the air for twelve hours and then planted in a 30% saturated soil. In cutting through the 100 grains taken at random from the 200 treated, it very soon became apparent that in most cases a selection from external appearances alone could be made. This was attempted before planting the remaining 100 of the treated grains. From external examination these 100 grains were divided into three groups: first, those seeming to be free from the colored oil; second, those showing slight traces of it; and third, those in which the embryos were deeply stained. The grains of these groups were planted in separate rows in the culture pans and were kept under identical conditions. Of the 76 grains of group one, all germinated and produced normal seedlings. Nine out of 14 of the second group, germinated but produced seedlings showing greater or less injury. One grain from group three germinated, the seedling being decidedly weak.

It has already been stated that the grains were carefully selected before they were immersed in the oil. Any having visible defects were rejected, but no selection was made after removing the corn from the oil, with the following exception: April 20, a quantity of the

Champion White Pearl was removed from the kerosene in which it had been placed February 6—76 days' immersion—and 50 grains were selected from it which seemed from external appearances to be free from kerosene. These grains were exposed to the air for ten days and then planted in a 30% saturated soil. Forty-nine, or 98% of them, developed normal seedlings. From these results it is apparent that an almost perfect germination can be secured by selecting grains showing no traces of stain in the embryos. The selection of grains with membranes slightly permeable to the colored oil can by no means so easily be made. The structures at the tips of the grains always take up the oil readily and it spreads for some distance from them, giving the appearance of stain within the embryos when in reality it is entirely superficial. The absence of Sudan III in the grain does not necessarily mean the absence of kerosene, since the membranes may be semi-permeable. A number of experiments were undertaken to test this assumption.

Grains immersed in Sudan III kerosene for long periods and free from stain were carefully dissected, and the structures within the coats were tested by the picric acid methods of Schulz ('08), and Krauz ('09). The results were uniformly negative. It was found that tests by these reagents were not nearly so delicate as the sense of taste. In no case, however, could the presence of kerosene be detected in unstained grains. On the other hand, it could be readily detected in grains which had been but slightly stained with Sudan III. It should be here stated that the above holds true only for air-dry grains.

These facts indicate very clearly that the kerosene enters some of the grains and is excluded from others. Whether the membranes of the grains showing penetration had been mechanically injured or were of different physical structure has not been determined. In either case the result would be the same. Undoubtedly there are many opportunities for mechanical injuries, but the fact that the number of grains exhibiting a penetration of the kerosene increases with the time of immersion would indicate that the membranes are not uniformly impermeable.

Membranes of widely different properties are not uncommon in seeds of the same kind. Many cases of delayed germination are attributed to this peculiarity [see Crocker ('06); Hänlein ('80); Nobbe and Hänlein ('77)]. It is not unlikely that the membranes of the corn kernel are sufficiently different in their organization or development to permit a rather wide variation in their permeability to kerosene.

A number of interesting studies on the physical properties of plant membranes have appeared recently. Brown ('07 and '09) found the "seed" of *Hordeum vulgare* to be enclosed in a semi-permeable membrane. He found the aleurone layer of *Hordeum vulgarė* to contain a pigment which serves as an indicator for acids and alkalis. This was not only a very interesting discovery but one which materially aided in the successful conduct of his work. He learned that the intact membranes of *H. vulgare* are impermeable to sulphuric acid; consequently when, in the presence of this acid, the purple pigment changed to a pink color it indicated imperfect membranes. Thus it was possible for him to select "seed" with intact membranes for experimental purposes. From all indications, Sudan III is just as efficient for determining imperfections in the membranes of *Zea* as are the color reactions described by Brown.

Schröder ('11), using Brown's methods, found the same kind of semi-permeable membranes in wheat. More recently Shull ('13), has made similar studies on the tests of *Xanthium glabratum* and demonstrated selective semi-permeability like that found in *Hordeum.*

5. MUTILATED MEMBRANES

To determine the toxic action of kerosene on the embryo, the outer membranes were punctured at several places and also removed. The following tables (Series B, tables 5 to 10 inclusive) give the results.

SERIES B

Tables 5 to 10 inclusive. Effects of kerosene on grains with membranes punctured before immersion. Champion White Pearl Corn germinated in a 25% saturated soil.

TABLE 5. NORMAL GRAINS (CONTROL)

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	0	100	100
2	50	10 days	"	50	0	100	100
3	50	15 days	"	50	2	100	96
4	50	20 days	"	50	2	100	96
5	50	25 days	"	46	3	92	86
6	50	35 days	"	44	6	88	76
7	50	50 days	"	40	4	80	72
8	50	75 days	"	38	1	76	74

TABLE 6. PEDICLE REMOVED

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	0	100	100
2	50	10 days	"	50	0	100	100
3	50	15 days	"	50	0	100	100
4	50	20 days	"	50	1	100	98
5	50	25 days	"	50	0	100	100
6	50	35 days	"	50	0	100	96
7	50	50 days	"	50	1	100	98
8	50	75 days	"	47		94	94

Series B—*Concluded*

Table 7. Pericarp Punctured at Distal End of Coleoptile

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	30	100	40*
2	50	10 days	”	44	38	88	12
3	50	15 days	”	34	32	68	04†
4	50	20 days	”	30	28	60	04†
5	50	25 days	”	22	22	44	00
6	50	35 days	”	6	5	12	02
7	50	50 days	”	0		00	00
8	50	75 days	”	1	1	02	00

*Not so vigorous as control.
†Retarded and decidedly weak.

Table 8. Membranes Lying within the Pedicle Punctured

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	0	100	100*
2	50	10 days	”	50	0	100	100*
3	50	15 days	”	48	40	96	16†
4	50	20 days	”	50	42	100	16†
5	50	25 days	”	32	32	64	00
6	50	35 days	”	30	28	60	04
7	50	50 days	”	20	20	40	00
8	50	75 days	”	0		00	00

*Slightly retarded.
†Retarded and weak.

Table 9. Pericarp Removed

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	12	100	76*
2	50	10 days	”	50	10	100	80*
3	50	15 days	”	36	20	72	32*
4	50	20 days	”	30	30	60	00
5	50	25 days	”	16	16	32	00
6	50	35 days	”	10	10	20	00
7	50	50 days	”	2	2	04	00
8	50	75 days	”	0		00	00

*Retarded and weaker than control.

Table 10. Pericarp Removed

Trial	No. of grains	Exposed to dry air	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	50	5	100	90
2	50	10 days	48	7	96	82
3	50	15 days	46	?*	92	?*
4	50	20 days	45	?*	90	?*
5	50	25 days	46	?*	92	?*
6	50	35 days	40	?*	80	?*
7	50	50 days	48	?*	96	?*
8	50	75 days	46	?*	92	?*

*These seedlings were uniformly weak. It could not be told at the time of observation whether they would recover or not.

The fact that the pericarp is greatly modified at the tip of the grain—the pedicle—into a very porous vascular tissue, introduces a factor that greatly increases the difficulty in a study of the membranes. At this point, however, the pericarp is reinforced within by a compact remnant of the nucellus which, when perfect, effectively prevents the oil from penetrating the grains. There can be no question but that this is the usual point of ingress into those grains in which the embryos are stained. The colored oil invariably makes its appearance here and gradually passes up through the embryo. That the pedicle itself is not only valueless for excluding the oil but that it is the source of positive injury is shown in Table 6. The pedicles were carefully broken off from these grains before immersing them in kerosene, and it was found that a higher rate of germination resulted from grains so treated. It is altogether likely that the spongy tissue of the pedicle, by retaining rather large quantities of the kerosene, is responsible for a decrease in germination when it is not removed. The oil remaining in or on the grains seems to be absorbed and carried to the regions of growth as soon as the growth processes are initiated. If the amount is beyond a certain limit, injury is produced.

The effects of removing the pedicle and slightly puncturing the membranes within it are shown in Table 8. The best place to puncture the pericarp without injuring the embryo is near the distal end of the coleoptile where, in the process of maturing, a small wrinkle is formed in a rather large proportion of the grains. The membranes at this point can readily be ruptured with a needle without the slightest injury to the underlying parts.

The effects of thus puncturing the coats (Table 7) are in a general way comparable to those secured by puncturing the membranes lying within the pedicle. In the latter case, however, the grains did not show the injurious effects as quickly as in the former. In both cases the colored oil penetrated the embryos in sufficient quantities to be plainly visible in twenty-four hours. It is important to note that, excepting a slight retardation, 100% of the grains in Table 8 continued to produce normal seedlings after immersion in kerosene for a period of ten days. This shows that the presence of a limited amount of oil in the embryo is not necessarily injurious.

By soaking the grains in tepid water for ten minutes the entire pericarp, including the pedicle, is very easily removed without injury to the parts within. After thus removing it, the grains were dried at room temperature for five days and then immersed in kerosene. The results (Table 9) correspond closely to those obtained in the experiments with punctured membranes and show that a punctured pericarp is equivalent to its removal. In either case the dormant grains are killed within a comparatively short time (75 days).

It will be noticed that grains with the pericarp removed but not otherwise treated (Table 10) retained the power of germination to a fairly high degree for the time indicated in the table; but such grains after ten days' exposure to the dry air of a steam-heated room produced seedlings that were uniformly weak. This indicates that these membranes play a very important rôle in preserving the vitality of the grains.

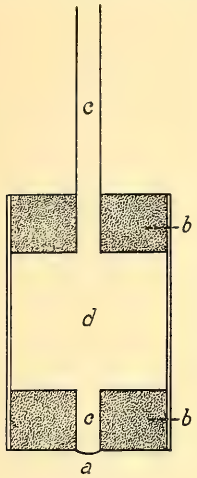

Fig. 1. Apparatus used in testing directly the permeability of the pericarp of *Zea*: *a*, membrane; *bb*, rubber stopper; *cc*, glass tube; *d*, receptacle for liquid to be tested.

The dry pericarp, not including the pedicle, was shown to be impermeable to kerosene in another way. A large grain of the Champion White Pearl furnishes a membrane fully one-half inch in diameter. It is easily removed after soaking the grain for a few minutes in warm water. After drying, it can be cemented over the end of a glass tube for use either as a barometer or as an osmometer. This simple piece of apparatus was, as far as I know, first devised by Becquerel ('07) in his studies on the permeability of seed coats to certain gases. Shull ('13) also used it with success in demonstrating the semi-permeability of the testa of *Xanthium*. Adapted for my work, the apparatus was constructed as shown in the accompanying sketch.

Considerable difficulty was experienced in finding a cement not soluble in kerosene. Sealing-wax, such as is used by express companies for sealing valuable packages, was finally found to serve the purpose admirably. The rubber stopper at the end of the glass cylinder serves as a foundation to which the membrane is cemented. The small glass tube (Fig. 1, *cc*) was allowed to protrude three or four millimeters through the perforated rubber stopper. A layer of wax equal in thickness to the protruding portion of the tube was then applied and the edges of the membrane were pressed into it while it was still soft. More wax was then applied to make the seal perfect. The central portion of the membrane over the end of the small glass tube was left entirely free from wax.

Such an apparatus was set up March 7, with kerosene on one side of the membrane and with plaster of Paris as an absorbent on the other. At the present time (May 6) the wax is holding perfectly and there has been no trace of oil passed through the membrane. A similar apparatus was set up as a barometer November 29 and has supported a mercury column representing a complete atmospheric pressure since that time (5 mos., 7 days). The mercury rises and falls with the changes in atmospheric conditions, but no fall attributable to the penetration of air through the membrane has taken place.

No effort has been made to extend these studies beyond the limits indicated in the title of the paper, but as a matter of interest the apparatus was set up as an osmometer with a saturated solution of sodium chloride on the inside of the membrane and distilled water on the outside. The contents of the upper tube, a cross-section of which had the same area as the exposed membrane, rose at the rate of 4½ cm. a day for four days. Before the rise had ceased the liquid outside the membrane was tested with silver nitrate for the presence of sodium chloride. The test showed the presence of the salt in large quantities.

From these experiments it may be concluded that under the conditions described the membrane is impermeable to kerosene and to atmospheric gases, but that it is permeable to sodium chloride.

6. MOISTURE CONTENT OF THE GRAINS

The data thus far discussed pertain to the corn which was thoroughly air-dried before it was immersed in kerosene. The following experiments show the effects of similar treatments on grains containing different amounts of moisture at the time of immersion.

Series C

Tables 11 to 14 inclusive. The effects of different amounts of water in the grains at time of immersion in kerosene. Champion White Pearl Corn germinated in a 25% saturated soil.

Table 11. Water in Grains Desiccated to Constant Weight at 100° C.

Samples (50 grains)	Original weight	Dry weight	Ratio of water to dry weight
Old corn....................	24.040	23.523	2.19%
New corn....................	26.832	22.112	21.30%
New corn soaked 1 hr........	28.185	22.468	25.44%

Table 12. Old Corn. Water Content Equivalent to 2.19 per cent. of Dry Weight

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	0	100	100
2	50	10 days	”	50	0	100	100
3	50	15 days	”	48	4	96	88
4	50	20 days	”	46	2	92	88

SERIES C—*Concluded*

TABLE 13. NEW CORN. WATER CONTENT EQUIVALENT TO 21.3 PER CENT. OF DRY WEIGHT

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	46	4	92	84
2	50	10 days	”	36	8	72	56
3	50	15 days	”	8	8	16	00
4	50	20 days	”	0		00	00

TABLE 14. NEW CORN SOAKED IN WATER 1 HR. WATER CONTENT EQUIVALENT TO 25.44 PER CENT. OF DRY WEIGHT

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	42	1	84	82
2	50	10 days	”	10	5	20	10
3	50	15 days	”	0	0	00	00

The amount of water contained in the respective samples used, as shown by desiccation to constant weight at 100 degrees C., is recorded in Table 11. The old corn was harvested one year previous and had been stored in the dry rooms of the laboratory for approximately six months. The new corn had just been harvested, but was fully mature and sound in every way. It will be noted that the old corn contained an amount of water equivalent to 2.19% of the constant weight at 100 degrees C.; the new corn, 21.3%; and the new corn soaked in water for one hour, 25.44%. Under normal conditions both the old corn and the new corn germinated perfectly.

The corn containing water in the amounts indicated above was immersed in kerosene and tested for viability at intervals of five days. The results are brought together in tables 12, 13, and 14. A glance at these tables shows that after a period of more than five days' immersion in kerosene the injuries are very decidedly increased in the grains of high water content. One hundred per cent. of the dry grains germinated after ten days' immersion in kerosene, while the germination of the new corn and the new corn soaked in water dropped to 36 and 10% respectively. Immersion in kerosene for twenty days proved fatal to all the corn of each lot containing the higher percentages of water. The air-dry corn, after an equal period of immersion, gave 92% germination and 88% normal growth.

Since the per cent. of germination falls so rapidly in corn not fully dry and in corn soaked in water for a short time, it seems evident that some physical change of the investing membranes takes place on moistening, and that they become more readily permeable to kerosene.

After twenty days' immersion in Sudan III kerosene, and after germination tests had proven all the grains dead, the remaining grains of the new corn were carefully examined to determine the number showing penetration of the stain. Of the 161 grains, 24, or approximately 15%, were stained. Twenty-four days later—74 days' immersion—the number had increased to 45, or 25%. If one should attempt to judge the viability of the grains by the presence or absence of the stain, as was done so effectively in the dry grains, the rate of germination should be approximately 75%. Both stained and unstained grains, however, had lost all power of germination and the presence of kerosene was easily demonstrated in both. The conclusion naturally follows that the membranes of the moist grains permit the penetration of the kerosene, but that they effectively prevent the passage of the Sudan III. The percentage of grains stained by Sudan III was approximately the same as in the dry grains. This supports the view previously expressed; namely, that the stained embryo is an indication of imperfect membranes.

7. VARIATIONS IN SOIL MOISTURE

That some grains of corn bear immersion for a period of eight years in kerosene is experimentally proven. This, however, is not true of all grains of like origin subjected to similar treatment. In every sample taken at random a certain percentage of the grains fail to germinate after a comparatively short period of immersion. By means of the Sudan III it has been conclusively established that a limited number of grains of a random sample are stained and that these eventually fail to germinate even under the most favorable conditions. Death in these instances is due to the toxic action of the kerosene on the dormant embryo. Since it has been shown that the dry membranes are impermeable or only slightly permeable to kerosene, the presence of the oil within the membranes, in sufficient quantities to cause death, is attributable to imperfect membranes. The presence of small quantities of kerosene within the grain, however, does not necessarily prove injurious. Grains immersed in kerosene for the same periods of time give very unlike results when placed under different conditions for germination. It was found that in the presence of abundant moisture the injurious effects of the kerosene treatment are especially marked. In the experiments (Series A) in which grains, similarly treated with kerosene, were placed in soils with different moisture content, this injury was clearly brought out. When the amount of water in the soil was reduced from 30% saturation (Series A, Table 1) to 25% saturation (Series B, Table 5) the per cent. of germination was increased and the growth of the seedlings was more nearly normal; but when the water content of the soil was increased to 50 or 75% of saturation (Series A, Tables 2 and 3) the per cent. of germination was markedly decreased and the subsequent growth of many of the seedlings abnormal.

The germination and growth of grains immersed in Sudan III kerosene but unstained is normal in 25 and 30% saturated soils. The slightly stained grains, that is those containing small quantities of kerosene, frequently produced normal seedlings when the water content of the soil did not exceed 25% saturation. In 30% saturated soil the per cent. of normal growth of these seedlings was greatly reduced. In soils of 50 and 75% saturation all grains showing the slightest penetration were killed, as were also a considerable number in which the presence of oil could not be detected from external examination.

Traces of kerosene are always present when once the grains have been immersed in it. This is shown by the decreased germination in soils of high water content and also by other and more direct evidences. Grains immersed for comparatively short periods retain the taste of the oil after six months' exposure to dry air at room temperature. Because of the varying moisture content of the corn, and possibly changes due to the presence of the kerosene, the exact amount of oil taken up and retained could not be accurately determined. Quantitative evidence, though desirable, was not necessary to show that a considerable residue remained after volatilization had been carried to the limit used in this work. The question, then, of the disposition of the oil or its residues in those cases in which no injurious effects are produced becomes important.

Schmidt ('91), in his studies on the translocation of oils in the living plant, devised a method by which he succeeded in directly introducing almond oil, cocoa butter, and other oils into the tissues of the stem. He showed that these oils were taken up and moved with considerable rapidity through both stem and leaf. He concluded that both neutral oils and fatty acids could be taken up by the growing plant, saponified and emulsified in a manner similar to that carried on in the animal organism.

Kryz ('09 and '13), investigating the effects on plants of oils used as insecticides, treated *Impatiens* with vaseline, and *Datura* and *Alisma* with kerosene. In the latter case he planted the seeds in flower-pots containing garden soil and sprinkled the soil with a 5% solution of the oil both before germination and after the plants had reached considerable size. He showed that the oil was taken up and carried through the vascular tissues to the leaves, where it was stored in quantities sufficiently large to make its presence easily determinable. Unfortunately Kryz continued the treatment until the plants were killed. He seems not to have paid any attention to the power of recovery of the plant from injuries not at once fatal.

These investigations led me to believe that under favorable conditions a limited amount of kerosene might be absorbed and disposed of, without injury, by the growing corn seedling. Observations confirmed this belief. The coleoptiles of seedlings grown from grains immersed in colored oil frequently showed the red stain. In soils of low moisture content these seedlings developed normally, while in soils of high moisture content they were either killed or showed pronounced injury. Numerous attempts were made to demonstrate the presence of the oil in the tissues. Sections were treated with Sudan III, alkannin, and picric acid benzol (5), but because of the large amount of oil normally present in the structures of the young corn seedling and the very small amount of kerosene which ordinarily is present, the results were not successful. No satisfactory test for demonstrating the presence of kerosene in very small quantities has been found.

The experiments of Kryz were repeated in a modified form and his results confirmed. Corn seedlings were grown on filter-paper so that the roots penetrated the paper and entered soil contained in a pot below. When the seedlings were about three inches tall, from 1 to 3 drops of Sudan III kerosene were applied to the old grains at the base of the seedlings. A drop was equal to one-fiftieth cubic centimeter. In a few minutes the stain showed prominently in the stems of the seedlings and eventually reached the leaves in quantities sufficiently large to be plainly visible to the naked eye. All the seedlings treated with three drops died within five days after the treatment. The majority of the seedlings treated with one and two drops recovered. The amount of oil disposed of was certainly many times as much as could be retained in the dry grains immersed in oil and afterwards treated to eliminate it. It is apparent that the older seedlings can dispose of a much greater amount of oil than the younger ones.

It is evident that within certain limits the seedlings are not injured by the oil present at the time of planting provided growth is initiated in the presence of a minimum amount of water. The small quantities of kerosene are toxic in proportion to the increase of the moisture content of the soil. In the 50 and 75% saturated soils the dormant period of the grain is always less than 36 hours, while in a 25% saturation the time is extended to approximately five days. This increase of time affords the seedling an opportunity to dispose of the oils much more slowly, and it does so without injurious effects.

8. OTHER OILS

In addition to the kerosene the effects of a number of other petroleum oils have been studied. At the present time only the initial results have been obtained. These results indicate that the injuries due to the penetration of the dormant grains by the oils are essentially the same as in the case of kerosene. The effects on the germinating grains, however, differ very widely. The more volatile oil (gasoline, Table 16) produces no more injury than does the kerosene. From present indications it seems probable that a high moisture content of the soil affects the grains immersed in the more volatile oils less than those immersed in kerosene. On the other hand, the injurious effects of the heavier oils on germinating grains in soils of either low or high moisture content are much more pronounced. The same means were employed for eliminating these oils from the grains after immersion as were used with the kerosene; viz., wiping the grains carefully with a towel and then exposing them to the air. The heavier oils do not volatilize as completely as the kerosene and gasoline do. The residues dry on the grains, producing a hard coating which prevents normal germination.

At present the trend of evidence tends to show that the grains bear immersion in the lighter oils without injury for much longer periods than in the heavier oils, and that the injurious after-effects of the latter are more pronounced than those of the former.

Series D

Tables 15 to 20 inclusive. Comparisons between kerosene and other petroleum oils. Champion White Pearl Corn germinated in a 25% saturated soil.

Table 15. Kerosene (Control)

Trial	No. of grains	Kerosene treatment	After treatment	No. germ.	No. injured	Per ct. germ.	Norm. growth
1	50	5 days	3 da. air	50	0	100	100
2	50	10 days	”	50	3	100	94
3	50	15 days	”	47	3	94	88
4	50	20 days	”	47	4	94	86
5	50	25 days	”	45	1	90	88
6	50	35 days	”	43	1	86	82
7	50	50 days	”	39	2	78	74
8	50	75 days	”	34	1	68	66

Table 16. Gasoline

Trial	No. of grains	Gasoline treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	49	1	98	96
2	50	10 days	”	49	3	98	92
3	50	15 days	”	47	2	94	90
4	50	20 days	”	47	3	94	88
5	50	25 days	”	45	1	90	88
6	50	35 days	”	42	1	84	82
7	50	50 days	”	40	0	80	80
8	50	75 days	”	37	1	74	72

Series D—*Concluded*

Table 17. Kansas Crude Oil

Trial	No. of grains	Oil treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	46	2	92	88
2	50	10 days	”	48	2	96	92
3	50	15 days	”	50	6	100	88
4	50	20 days	”	47	3	94	88
5	50	25 days	”	43	4	86	78
6	50	35 days	”	44	5	88	78
7	50	50 days	”	50	20	100	60
8	50	75 days	”	40	10	80	60

Table 18. Heavy Red Oil

Trial	No. of grains	Oil treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	50	0	100	100*
2	50	10 days	”	46	0	92	92*
3	50	15 days	”	40	5	80	70*
4	50	20 days	”	40	4	88	76*
5	50	25 days	”	40	3	80	74*
6	50	35 days	”	48	4	96	88*
7	50	50 days	”	40	28	80	24*
8	50	75 days	”	24	10	48	28*

*All seedlings decidedly retarded.

Table 19. Fuel Oil

Trial	No. of grains	Oil treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	48	2	96	92*
2	50	10 days	”	46	14	92	64*
3	50	15 days	”	46	18	92	56*
4	50	20 days	”	42	15	84	54*
5	50	25 days	”	44	14	88	60*
6	50	35 days	”	48	8	96	80*
7	50	50 days	”	42	26	84	32*
8	50	75 days	”	40	20	80	40*

*From 2 to 3 days retarded.

Table 20. Engine Oil

Trial	No. of grains	Oil treatment	After treatment	No. germ.	No. injured	Per ct. germ.	% Norm. growth
1	50	5 days	3 da. air	48	2	96	92*
2	50	10 days	”	48	20	96	56*
3	50	15 days	”	48	18	96	60*
4	50	20 days	”	45	16	90	58*
5	50	25 days	”	43	15	86	56*
6	50	35 days	”	40	12	80	56*
7	50	50 days	”	44	32	88	24*
8	50	75 days	”	40	24	80	32*

*Retarded 3 days and very uneven.

9. SUMMARY OF CONCLUSIONS

Grains of *Zea mais* may be immersed in kerosene for periods of ten to twenty days without injury if the optimum conditions for the germination and growth of such grains are provided. These conditions include the removal of the superficial oil from the grains and the presence of a minimum amount of water during germination and initial growth.

Injuries which occur to the dry grains immersed in kerosene for longer periods than above indicated are due to the penetration of the oil into the embryos through imperfect membranes.

The dry membranes covering the corn embryo, when perfect, are impermeable to kerosene and to Sudan III.

Some grains of *Zea mais* may be immersed in kerosene for eight years without injury to the dormant embryo.

The life of dormant grains, with membranes which have been mechanically injured, is destroyed within seventy-five days after immersion in kerosene.

Kerosene is injurious to the germinating grains in direct proportion to the length of time of immersion and to the increase of the water content of the soil above the minimum required for germination.

When moist grains are immersed in a solution of kerosene and Sudan III, the membranes are penetrated by the kerosene but not by the Sudan III. The membranes are, therefore, semi-permeable.

The germinating corn grain may absorb and dispose of a limited amount of kerosene without injury. The smaller the amount of water present during germination the larger the quantity of kerosene which can be disposed of. Older corn seedlings may dispose of comparatively large quantities of kerosene without injury.

It is not advisable to treat seed corn with kerosene unless the water content of the soil is under control.

The injurious effects of petroleum oils on germinating corn seem to vary inversely as the volatility of the respective oils.

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VITA

1873, Oct. 3.	Born in Stark County, Illinois.
1879—1887.	Attended country school in Penn township, Stark County, Illinois.
1887—1891.	Time divided between farm and country school, Stark County, Illinois.
1891—1892.	Student in High School, Bradford, Illinois.
1892—1894.	Teacher in country school, Stark County, Illinois.
1894—1896.	Student in Illinois State Normal University, Normal, Illinois.
1896—1897.	Principal, Public Schools, Diamond, Illinois.
1897—1899.	Student, Illinois State Normal University, Normal, Illinois. Graduated 1899.
1899—1901.	Principal, Public Schools, Golconda, Illinois.
1901—1903.	Principal, Public Schools, St. Anne, Illinois.
1903—1910.	Superintendent, Public Schools, Onarga, Illinois.
1910—1914.	Assistant in Botany, University of Illinois.

Degrees

1911. A.B., University of Illinois.
1912. A.M., University of Illinois.

PLATE XVI

Plants grown from grains of *Zea mais* immersed in kerosene from February 6, 1906, to February 6, 1914. On removal from the kerosene the grains were cleared of superficial oil by means of absorptive towels, and then washed vigorously for five minutes in chloroform and exposed to dry air at room temperature for five days. They were planted February 11, 1914, and this photograph was taken April 22, 1914.

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