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New scientific names are printed in full-faced type

ERRATA FOR VOLUME VII

Page 49 line 27, for uredinospores read urediniospores.
Page 113, 114, and 115, for *Corticum* read *Corticium*.
Page 113 line 9 and page 114 line 4, for *ochraleucum* read *ochroleucum*.
Page 132 line 5, for *Cephaleurus* read *Cephaleuros*.
Page 134, for Illinois University read University of Illinois.
Page 184 line 7, for frum read from.
Page 193 line 1, for 30 read 50.
Page 208, section 6 of summary, line 3, for diseases read diseased.
Page 350, legend for figure 3 should read *Zygosporium oschioides*.
Page 369, 370, and 371, in table headings for uredinospores read urediniospores.
Page 376, table 1, column F, for 26° read 26.
Page 386 line 10, for Tomasski read Pomasski.
Page 390 line 12, strike out comma after water,
Page 418 line 1, after cane insert disease.

PHYTOPATHOLOGY

VOLUME VII NUMBER 1

FEBRUARY, 1917

JENS LUDWIG JENSEN
(1836–1904)

F. KØLPIN RAVN

WITH PORTRAIT, PLATE I

The introduction of "hot-water treatment" is one of the most important steps forward in practical plant pathology, for it means a new principle both from a theoretical as well as from a practical point of view. Therefore it will surely interest the readers of PHYTOPATHOLOGY to become acquainted with the originator of the method, because in it we find an example of progress due to a man from practical life, who had scientific qualifications as well, and the ability to carry on independent research.

Jens Ludwig Jensen was born January 9, 1836, near the little town of Odder in the Danish province of Jutland, where his father was forester. In 1855 he passed a normal school examination and later supplemented his education by studying natural science. He taught school until 1872. In 1868 he started a weekly agricultural magazine, of which he remained the editor until 1880, and from 1879 until his death, he was the publisher of a widely circulating advertiser.

In 1872 Jensen resigned his position as teacher, and together with a colleague started a company for selling scientifically tested seed. This has had a great influence on the development of modern agriculture in Denmark. He took part in the daily routine of the business until 1881, and remained one of its directors until 1896.

Jensen never held any official government position but he managed to arrange his practical affairs in such a way that he could spend much time in study, and in 1881 he organized this work as a private statistical institute which he called Bureau Ceres. Its object was a systematic collecting of observations made in practical agriculture and an experimenting both in field and in the laboratory, which he had equipped in his private home. Although many of Jensen's methods were primitive,

yet his work is so exhaustive and original that his investigations of potato-late-blight fungus (*Phytophthora infestans*) and smut in cereals are among the most important contributions to our knowledge of these diseases.

Jensen's incentive for investigating the potato disease was the work done by his company in introducing new varieties of potatoes into Denmark. In the years 1878–1881 more than one thousand experiments were made in all parts of the country and while inspecting these Jensen had an opportunity of studying the appearance of the disease under various conditions. This suggested infection experiments in which he studied the spread of the infection from the leaves to the tubers and more particularly the ability of the soil to retain the spores of the fungus by filtration.

In 1882 Jensen published his experiments which resulted in the development of a method, "protective moulding," to prevent the tubers from infection. He discovered, too, that by postponing digging for two weeks after the wilting of the top, tubers otherwise subject to late attacks of the disease might be kept from infection.

In June 1882 Jensen began his important experiments concerning the influence of temperature on the development of the fungus. He discovered among other things, that this could not grow in a temperature of under 5°C. or over 24°C. Therefore the storage temperature of potatoes during the winter should never go higher than 5°C. These experiments gave Jensen the clue to understanding why the fungus attack is severe only in temperate climates, and he formed an hypothesis for explaining why the potato disease was not observed in North America and Europe before 1840. First then was the development in the means of transportation such that the potatoes from the plateau regions of South America, the original home of the potato and the potato fungus, might be carried so quickly through the tropical zone that the hyphae in the tubers did not die on the way.

In October 1882 Jensen succeeded in demonstrating that the hyphae and spores which were found in and on the diseased tubers could be killed by applying a temperature of 40°C. for four hours to the potatoes without affecting their germinating power. The heat must be applied as hot air for the germinating power suffered too much when the tubers were immersed in hot water.

In 1883-1884 Jensen published his method for disinfecting seed potatoes by heat. When this was used it was possible to prevent the primary attacks of the disease and to delay the secondary attacks at least one to two weeks.

Until bordeaux mixture appeared for the first time in 1886 as a preventive of disease, Jensen's system for fighting the potato fungus was the

best established method both practically and scientifically. In 1886 his work received recognition from La Société Nationale d'Agriculture de France, and he was awarded the large gold medal of the society.

In 1885 Jensen began to investigate smut in cereals, and by means of cross-inoculation experiments he succeeded in demonstrating that the old well-known species *Ustilago segetum* should be divided into four varieties which he named, *tritici*, *avenæ*, *hordei nuda*, and *hordei tecta*. The distinctiveness of these varieties was later confirmed by the mycological research of Rostrup, Brefeld, and Kellermann and Swingle. These infection experiments made it appear most probable that smut on barley and oats was caused by infection during the blossom period and that the smut spores (or hyphae) are to be found under the glumes in the ripe kernels. The importance of the rôle played by infected seed was further demonstrated by experiments proving the impossibility of infection through the soil and manure.

In 1887 and 1888 the most important experiments in disinfecting seed were published. Jensen compared the chemical remedies proposed by others (copper sulphate, sulphuric acid, quicklime, salt) with the hot water treatment he himself had found. He first applied hot air during a long period as for the potato fungus, but the results were for the most part unsatisfactory. However, an experiment made in the summer of 1887 in treating oats with hot water, circa 55°C. for five minutes, resulted in killing the smut without affecting the germinating power of the oats. This experiment forms the starting point for the development of the "Jensen hot-water treatment."

Jensen now made this interesting observation: Smut in barley is not to be killed by the same treatment as smut in oats, but if damp barley seed has been subjected to a temperature of 53°C. for five hours in a corked bottle the smut disappears. Jensen explained this phenomenon by supposing that a five minutes' immersion in hot water was insufficient to moisten the smut germs hidden in the seed and that they therefore were treated in a dry condition which could not occasion their death. By the slow heating of wet barley the moisture had time to penetrate and soften the smut germs and these were therefore killed by the temperature applied. If this were true Jensen thought that smut on barley could be prevented by soaking the seed in cold water and then applying the usual hot-water treatment for five minutes. Experiments made in 1888 proved the truth of this theory.

As we see, Jensen had now discovered two variations of a method according to which all forms of smut on cereals might be fought. He also proved that hot-water treatment could be used for smut on Bromus and Arrhenatherum and that it might be used as a preventive measure against certain types of damping-off in sugar beets and mangels.

Jensen's method won and deserved much recognition both in Denmark and abroad. However, it was a great disappointment to him to see that many farms which had introduced the method abandoned it later. It was too complicated for general practical use where no steam was available. Nor was he successful in his attempt to form a company for building a factory for applying the hot-water treatment of cereals on a commercial scale.

After this Jensen discontinued his work with the hot-water treatment and devoted himself to experiments with chemical remedies. Among these he preferred potassium sulphide, first tested by Kellermann and Swingle. This substance was the main ingredient in the so-called Ceres-powder, manufactured by Jensen and placed on the market in 1895. The last years of his life until his death, August 10, 1904, were largely devoted to experiments with this remedy and agitation to bring it into practical use.

During the years since Jensen's death the hot-water treatment has again come to the front. In connection with many of the Danish dairies and breweries, cooperative institutions have been established for disinfecting seed with hot water. During the past year several seed firms have built factory plants for the hot-water treatment in combination with a plant for drying the seed. The seed which has been thus treated is sold with a guarantee for its freedom from smut and leaf-stripe disease.

THE PENETRATION OF FOREIGN SUBSTANCES INTRODUCED INTO TREES

W. H. RANKIN

WITH ONE FIGURE IN THE TEXT

Meyer[1] in 1808 succeeded in introducing a dyeing liquid into the roots of a small tree by cutting the stem and immersing the upper part of it in the liquid. The solution penetrated after some time into all the roots with the exception of their tips and the slenderest rootlets.

Boucherie[2] about 1840 patented his method of preserving timber for building purposes. He made a shallow groove around the tree and covered it with a belt. The space under the cloth was then connected with a barrel containing the preserving liquid. The solution was absorbed and ascended to the branches and leaves. Later, he modified his method. A canal two centimeters in diameter was made through the stem and from the latter cuts were made with a saw on both sides as far as possible without allowing the tree to fall over. The liquid was distributed up and down the stem. The area saturated, however, decreased rapidly in breadth in the downward direction. He states that the best season for thorough penetration by this method is autumn. It is doubtful as to the meaning of the expression "thorough penetration" since he further states that if there are hard knots or rotten spots at the base of the tree the whole strip of wood above them did not become saturated at all and the same was true with the central part of the core of deciduous trees.

Hartig[3] introduced colored solutions into the growing stems of trees. He bored two holes at right angles to each other in the trunk and introduced the colored solution into them. It was carried to the top of the tree but in transverse sections made of the trunk the coloring of the wood was not uniform. Only those vessels directly above the canals were colored, forming a cross in the sections.

[1] Meyer, J. C. F. Naturgetreue Darstellung der Entwickelung, Ausbildung und des Wachsthums der Pflanzen und der Bewegung und Functionen ihrer Säfte. Leipzig. 1808.

[2] Boucherie, M. A. Mémoire sur la conservation des bois. Annales de chemie et physyque **74**: 113–157. 1840.

—— Nouvelles recherches sur la conservation des bois. Comptes Rendus **12**: 337–339. 1841.

[3] Hartig, T. (Discussed by Shevyrev 1903: 6–7, but the direct citation to Hartig is not given.)

Sachs[4] in experiments on the rate of ascent of sap in woody plants used lithium nitrate. He allowed the plants to absorb this through the roots and found the lithium present at intervals along the stem and at the tips of the branches. He performed laboratory experiments in which he showed that such a substance as lithium would progress in the stem almost as rapidly as water itself, while solutions which dye the cell walls along its upward course do not rise nearly so rapidly nor so far, since they are largely filtered out.

Shevyrev[5] was the first to utilize the original negative tension of gases in the tree as a force for distributing the introduced foreign substance. He attributes the failure of Hartig and others to get penetration, except immediately above the incised vessels, to the neglect of this factor. His method was to attach a funnel or half-funnel to the tree, fill it with water and then make an opening in the tree with a chisel or auger, underneath the surface of the liquid. As a modification of this he devised a metal tube which was previously connected with a reservoir containing the feeding solution. This was forced at one end into the bark. The other end was closed with a rubber stopper through which a bit was inserted. Thus when the boring was done the solution from the reservoir penetrated immediately into the wound and the sucking power due to the negative tension of the gases in the tree was utilized to pull in the solution. He states that by this method the dye permeates not only the aerial but also the radicate parts of the plant. As to the results, he states: "The absorbed liquid had risen to the top and colored all the veins of the leaves and even the veins of the berries on the grape vine. The dye could be detected five feet below the surface on all of the roots of the birch, apple and ash trees. Thus the first part of the problem is solved; we can introduce a liquid in a desired quantity into all parts of a tree." Further he states: "The vessels incised in the liquid, absorbed and distributed it to all parts of a living tree. Only the pithy, dead portion of the tree was not saturated with the liquid although its absorption by the rays occurred (with an oak). The liquid entered the roots as well as the leaves, twigs and fruit." The data given for each tree fed do not indicate that he obtained anything more than the saturation of the sap wood and bark

[4] Sachs, J. Ein Beitrag zur Kenntniss des aufsteigenden Saftstrom in transpirirenden Pflanzen. Arb. bot. Inst. Wurzburg **2**: 148-184. 1878.

[5] Shevyrev, Ivan. (Extraradicate nutrition of diseased trees with the aim of curing them and destroying their parasites.) M. Z. and G. I. Forestry Dept. Report to Forestry Department about injurious insects, pp. 1-51. 1903. (Reprinted from Selsk. Khoz. i. Lyesov. 1903: 58-103.)

(Supplements to the "Extraradicate nutrition of diseased trees with the aim of curing them and destroying their parasites".) Zemledeltcheskala gazeta (Agricultural Gazette) Nos. 3, 4, 5, 6. 1904. (Reprint consulted, pp. 1-13).

of root and stem and the leaves, for he mentions specifically that the medullary rays in the case of a single oak were colored.

Roth,[6] Goff,[7] Mangin,[8] Mokrzecki,[9] Bolley,[10] Simon,[11] Fron,[12] and others have fed trees with various types of solutions, including colored and nutritive substances. The majority of them have used nutritive salts or poisons in anticipation of curing physiologic ailments or inhibiting plant pathogenes and insects. No accurate data are given on the penetration except that the solutions in many cases were found to reach the leaves and some obtained penetration of the roots.

CHOICE OF SUBSTANCES FOR EXPERIMENT

It seems from the very nature of colored solutions, such as methyl blue and eosin, that they would not be suitable for determining accurately the greatest possible penetration obtainable by introducing a foreign substance. The staining quality is very helpful in tracing the rate and distance which the substance has advanced, but at the same time much resistance must be encountered by such substances and finally much of the original quantity absorbed will be adsorbed, filtered out and chemically united with the different plant parts which it will stain. The utilization of substances such as the lithium salts overcomes these disadvantages although the actual ascent is not visible. The salts of lithium are for the most part soluble in water, they are not used up rapidly in the metabolic processes of the plant and most important of all the minutest trace can be detected with the spectroscope. Lithium nitrate in solution was used in the experiments reported below.

[6] Roth, Carl. (A method for artificially feeding trees.) Chem. Ztg. **20**: 344–345, fig. 2. 1896.

[7] Goff, E. S. The application of artificial root pressure to recently transplanted trees. Wisconsin Agr. Exp. Sta. Ann. Rept. **14**: 272–282, fig. 4. 1897.

[8] Mangin, L. Sur la nutrition et la defense de la vigne par injection. Jour. Agr. Prat. **1898**: 918–920.

[9] Mokrzecki, S. A. (A new method of healing and nourishing trees.) Vyestvik Tavr. Zenistvo. nos. 11 and 12. 1903.

—— Über die innere Therapie der Pflanzen. Zeitschr. Pflanzenkr. **13**: 257–265, fig. 1–5. 1903.

[10] Bolley, H. L. (Artificial feeding of trees.) Report of the botanist. North Dakota Agr. Exp. Sta. Ann. Rept. **14**: 42–58. 1903. Ibid **15**: 33–65. 1904. Ibid **17**: 35. 1906.

[11] Simon, J. M. (Hypodermic injection in plants.) Jour. Soc. Nat. Hort. France. (Abs. in Gard. Chron. 3: **41**:8. 1907.)

[12] Fron, G. (Contributions to the study of the injection of nutrients into fruit trees.) Jour. Soc. Nat. Hort. France 4: **10**: 54–59, fig. 2. 1909.

FORCES AIDING DISTRIBUTION IN THE TREE

There seem to be three forces which must be depended upon for the rapid distribution of any foreign substance throughout a tree. (1) By taking advantage of the negative tension of the gases in a tree in the summer, when transpiration exceeds the intake of water through the roots, the solution containing the substance is quickly intromitted. Undoubtedly, the currents set up by supplying this ready access to a quantity of liquid serve to distribute the substance to a certain degree. (2) Most important of all, however, are the translocating streams of sap in the tree. The upward movement of raw sap will soon carry the substance to the leaves and the downward movement of the modified food materials in the phloem will undoubtedly carry the substance back down to all parts of the bark. The constant translocation of materials between such active cells as phloem parenchyma and medullary ray cells will serve to distribute it in time throughout these tissues and the downward movement of modified food into the roots would also be expected to ultimately carry the substance through the root tissues. (3) Except by diffusion, which is a very slow process, the only movements which can be counted on to distribute the substance in the heart wood are the translocation currents in the medullary rays and the alternate withdrawal and renewal of the water in the heart wood. The water in the center of the tree is said to act as a reserve supply upon which the tree draws during the day in dry weather. The normal water content of the heart wood is again restored at night. It would appear then that such an oscillation of currents might serve eventually to distribute the substance throughout the wood.

In other words there is no reason to believe that a foreign substance introduced into a tree cannot penetrate to all parts provided it possesses certain properties in itself.

METHOD OF FEEDING

Ten chestnut trees varying from two and one-half to nine inches in diameter were fed. The trees were growing in the forest and had small crowns. Shevyrev's methods with slight modifications were used in these experiments. A half-funnel was attached to the tree with putty. The funnel was then filled with water and a one-half inch hole was bored under the surface of the water with a brace and bit. The hole was bored to reach the center of the tree. A one-gallon bottle containing the lithium nitrate solution had previously been suspended so that the botton of the bottle was slightly higher than the hole in the trunk. The solution was then connected with the tree by means of a siphon, made of glass

and rubber tubing, one-quarter of an inch in diameter. In order to keep out air and prevent the leaking of the solution the apparatus illustrated by Rumbold[13] was used. The rubber siphon was connected to a short piece of glass tubing inserted through a one-inch rubber stopper. In attaching the siphon to the tree the solution was first started running and as the half-funnel was knocked from the tree the rubber stopper with the glass tubing of the siphon inserted was pressed against the tree so that it covered the hole. The end of the glass tubing was allowed to project into the hole about one inch. The rubber stopper was then held firmly against the tree by the use of wooden frames and a piece of No. 8 spring-steel wire. Thus the opening was perfectly sealed and after being once properly adjusted, needed no further attention. The glass siphon tube, reaching to the bottom of the bottle, was held in place by a loosely fitting stopper. By slightly raising this stopper a new supply of the solution could be poured into the bottle.

AMOUNT AND STRENGTH OF SOLUTION FED TO TREES

No attempt was made to keep careful records on the periodicity of the intake or to correlate it with any of the factors influencing the rate of intake. The accompanying table gives such data as were taken. Tree 1 had two days of clear, hot weather on July 15 and 16, when it absorbed

TABLE 1

Amount of lithium nitrate in liters of solution taken up by chestnut trees

TREE NUMBER	FIRST FEEDING			SECOND FEEDING		THIRD FEEDING	TOTAL	
	0.002 per cent			0.025 per cent	0.1 per cent	0.1 per cent	Liters	Grams
	Jy. 15–16	*Jy. 16*	*Jy. 16–20*	*Jy. 20–26*	*Jy. 26–A. 7*	*A. 13–0. 10*		
1	3	2	2	1	2	3	13	3.39
2		2	1.5	1.5	1	2	8	2.45
			Jy. 16-17	*Jy. 17–26*				
3		2	4	3.5	2	1	12.5	2.00
4					3	2	5	5.00
			Jy. 17–18	*Jy. 18–26*				
5		1	3	2	2	3	11	5.58
6					3	1	4	4.00
7						4	4	4.00
8						2	2	2.00
9						2.5	2.5	2.50

[13] Rumbold, Caroline. Report of the physiologist. Report of the Pennsylvania Chestnut Tree Blight Commission, July 1 to December 31, 1912, pp.45–47, figs. 39–49. 1913.

—— Methods of injecting trees. Phytopath. **5**: 225–229, pl. 13. 1915.

five liters of solution in twenty-six hours. Then followed a period of cloudy and cooler weather which caused a marked decrease in the amount absorbed by trees 1, 2, 3, and 5 during July 17 to 20. The largest amount of solution was absorbed immediately after attaching. Tree 2 absorbed two liters the first three hours and tree 3 absorbed five liters the first nineteen hours. However, the hole in tree 3 reached to decayed heart wood and the punky wood absorbed an unusual amount. The amount absorbed diminished rapidly after the first two days and in most cases practically ceased after the fifth or sixth day. Trees fed the second and third time did not take in as much as they did the first time. The increased strength of the solution used in the later feedings may have accounted for this. However, no detrimental effect on the tree was observed and the one-tenth per cent solution allowed the feeding of a sufficient amount in a shorter time. After the feeding on August 13, all the trees were allowed to stand until October 10 so that a chance was afforded for more complete distribution. The leaves were just beginning to fall when the trees were cut. A burning of the margins of the leaves occurred in the case of the smaller trees which had taken up as much lithium as some of the larger trees.

METHOD OF ANALYSIS OF TREES

The trees were cut as near to the ground as possible. Cross-sections about one-half inch thick were cut from the base, at the point of feeding and every ten feet up the trunk. A few leaves were taken from the tops of the trees. The sections were then seasoned. To obtain small blocks from these sections for spectroscopic analysis, a strip about one centimeter wide was sawed out along the diameter of each section. Where the bark was thick the cork layer was separated from one end of the strip, starting with the end which represented a point directly above or below the place of feeding. This was placed in a vial and labeled. Next the inner bark was split off from the wood. Then a small block about one-half centimeter thick representing the sapwood was cut from the strip. Similar blocks were cut from the strip at intervals of about two centimeters until the sapwood, inner bark and cork was reached at the other end of the strip. In this way it was considered that representative portions of the tree were obtained for analysis which would show rather accurately the penetration secured. The blocks were then incinerated in carefully cleaned crucibles in the bunsen flame. The ash was burned on a platinum needle in a colorless gas flame and the spectrum observed. The presence or absence of the red lithium line indicated whether or not the lithium had penetrated to the part of the tree represented by the block being analysed.

RESULTS

The results obtained were practically uniform. The blocks in which lithium was found are shown graphically in illustrations 1 to 9 in figure 1. In all of the trees except 3 and 6 complete penetration of the bark and sapwood was obtained at and above the point of feeding. In trees 3 and 6, for some reason, the lithium did not penetrate the sapwood on the side opposite the point of feeding. In all the trees except 1 and 4 the lithium had completely penetrated the sapwood and inner bark of the sections taken at the surface of the ground. In the case of trees 1 and 4 the solution had penetrated the bark and sapwood immediately below the point of feeding but did not appear in bark and sapwood on the opposite side. In trees 1 to 6, which varied in diameter from 5 to 9½ inches, the lithium had penetrated the heart wood only in a few cases, notably the basal sections of trees 1, 3, 4 and 5 and the section twenty feet above the point of feeding in the case of tree 2. Tree 4 showed more heart wood penetrated than any of the others. In this tree the heart wood was decayed where the basal and breast-high sections were taken and the bark and sapwood of the basal section on the side opposite to the point of feeding was not penetrated. However, in the first two sections above the point of feeding the lithium had penetrated for at least two centimeters inside of the sapwood in sound heart wood. In the case of the trees of smaller diameter (7, 8 and 9) complete penetration of the entire wood and bark was obtained. These trees (7, 8 and 9) were from 2⅛ to 3 inches in diameter and contained several layers of heart wood.

The leaves and twigs from the very tops of all the trees showed a large amount of lithium present.

CONCLUSIONS FROM DATA OBTAINED

From the above results it may be stated, therefore, that:

1. Lithium nitrate when fed to chestnut trees by Shevyrev's method penetrates to all places in the tree where there is an active translocation of food materials, that is, to all parts of the bark and sapwood above and below the point of feeding.

2. Complete penetration of the heart wood is obtained in trees less than three inches in diameter. In trees of greater diameter the process of penetration is slow and does not seemingly follow any definite rule.

CORNELL UNIVERSITY
ITHACA, NEW YORK

EXPLANATION OF FIGURE 1

The figures represent the strips of wood sawed out along the diameter of each cross-section cut from the trees. The blocks analyzed are shown in correct proportion as to size and position in the strip. Those blocks represented by shaded areas gave positive tests for lithium; those represented by white areas contained no lithium. All the figures are reproduced to a scale which equals one-fourth the size of the original sections.

The sets of sections are numbered according to the trees which they represent.

The sections from each tree are lettered as follows:

A. Section from base of tree (at ground)
B. Section at point of feeding (breast high)
C. Section ten feet above *B*
D. Section ten feet above *C*
E. Section ten feet above *D*
F. Section ten feet above *E*
G. Leaves taken from top of tree

Where three blocks are shown at the ends of the strips they represent from the outside inward, cork, green bark and sapwood respectively. Where only two are shown they represent bark and sapwood.

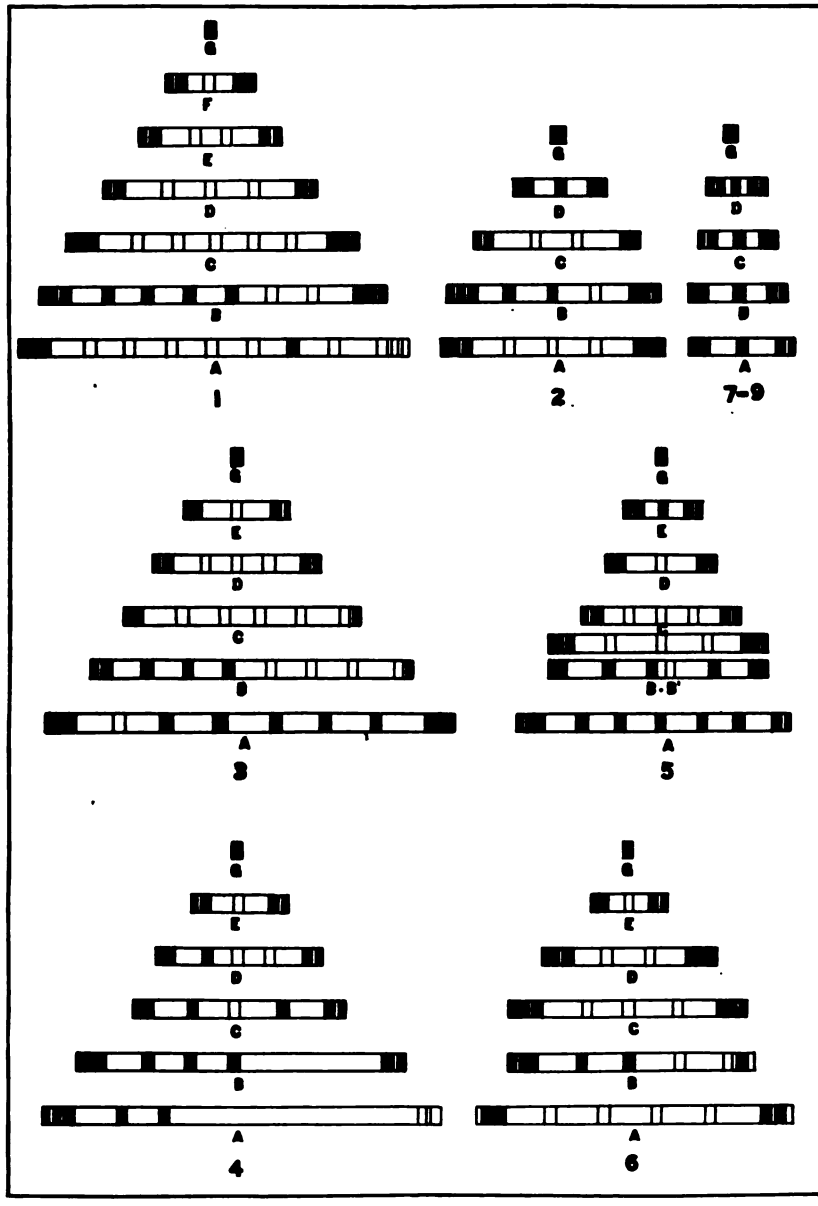

FIG. 1. DIAGRAMS ILLUSTRATING THE PENETRATION OF CHESTNUT TREES WITH LITHIUM NITRATE

THE GENERATION OF ALDEHYDES BY FUSARIUM CUBENSE

ELBERT C. LATHROP

The possibility of aldehyde occurrences in cultures of the organism from the Cuban banana disease was called to the attention of this laboratory by Dr. C. Rumbold because of the odor accompanying its growth. The possibility of aldehyde production by the fungus was further suggested by the work of the author on color production through interaction of aldehydes and certain plant constituents in connection with the investigation of humus bodies. One of the characteristics of the Cuban banana disease, which according to Smith[1] is due to the fungus, *Fusarium cubense*, is the purple, purple-brown, or blackish stain produced in the vascular bundles of the diseased banana plant. This Fusarium also reddens or purples various culture media. Numerous experiments carried on in this laboratory on the action of aldehydes of various chemical constitution in respect to their effect on plant growth have demonstrated that aldehydes are uniformly deleterious in action. The generation of aldehydes by *Fusarium cubense* might therefore account, at least in a measure, for its pathological action as well.

In regard to the generation of aldehydes by microorganisms Grey[2] has shown that acetaldehyde is a product of the action of *B. coli communis* on glucose under anaerobic conditions. That acetaldehyde is a product of the alcoholic fermentation by yeast was discovered by Roeser,[3] and more recently C. Neuberg and his co-workers have very fully studied the mechanism of this reaction. Neuberg and Hildesheimer[4] have shown, for example, that acetaldehyde is produced by the action of yeast on pyruvic acid, while Neuberg and Kerb[5] have been able to produce propionic aldehyde by the action of yeast on α-keto butyric acid.

That aldehydes are generated during the growth of *Fusarium cubense* on synthetic culture media was experimentally shown in the following way. Eleven 2-liter Erlenmeyer flasks, each containing about seven hundred cubic centimeters of Uschinsky's solution were sterilized in the

[1] Science **31**: 754 755. 1910.
[2] Biochem. Jour. **7**: 359 363. 1913.
[3] Ann. Inst. Pasteur **7**: 41. 1893.
[4] Zeit. physiol. Chem. **31**: 174. 1911.
[5] Zeit. physiol. Chem. **47**: 413 429. 1912.

autoclave and inoculated with a pure culture of *Fusarium cubense* on May 28, 1915. The flasks were set aside in a dark closet and the Fusarium was allowed to grow at room temperature until January 21, 1916, at the end of which time the Fusarium was still growing. The liquid culture media, which had darkened a little and which had taken on a slightly penetrating odor, was filtered from the sediment and growing Fusarium. The clear filtrate, alkaline in reaction, was slightly acidified with dilute sulfuric acid and the acid liquid was shaken out a number of times with ether which had been carefully freed from aldehydes. The aldehydes were removed from the combined ether extract by shaking with a freshly prepared, saturated solution of sodium bisulfite. The bisulfite solution was then acidified with dilute sulfuric acid, the sulfur dioxide removed by aeration under a long ice cold reflux condensor, and the volatile aldehydes, boiling under 75°, were separated from the solution by fractional distillation, and collected in about fifty cubic centimeters of ice cold distilled water. The aldehyde fraction so obtained was then treated with a little solid barium carbonate and redistilled in order to hold back any volatile acids. This distillate was tested for the presence of aldehydes.

A few cubic centimeters of the distillate when treated withSchiff's fuchsine aldehyde reagent gave a red color immediately. The distillate reduced ammoniacal silver nitrate solution, slowly in the cold, and very rapidly when gently warmed. The odor of the solution was that generally given by aldehydes, especially the lower aldehydes of the aliphatic series. On boiling a little of the solution with a strong solution of sodium hydroxide a pale yellow color was produced which disappeared on longer heating, and the odor of the solution strongly resembled that of lemon. This reaction is characteristic of propionic aldehyde, as distinguished from acetaldehyde or formaldehyde. All attempts to form the phenylhydrazone or the p-nitrophenylhydrazone compounds failed, probably owing to the small quantities of the aldehyde which had been obtained. The aldehyde in the remaining portion of the distillate was oxidized by means of dilute sulfuric acid and potassium permanganate solution to a volatile fatty acid, which was obtained in amounts too small to be identified by means of the formation of the metallic salts. By the method of obtaining the aldehyde the fatty acids obtainable by the oxidation with the acid permanganate mixture are limited to formic, acetic, propionic, butyric, isobutyric and trimethyl acetic acids. The odors of pure hot dilute solutions of formic, acetic, propionic and butyric acids are so sufficiently characteristic as to be readily distinguished from each other. Hot dilute solutions of these acids were then compared with the solution of the unknown acid and the odor of the pure propionic acid and of the unknown acid were so exactly similar that they could not be differentiated. This

would indicate that the volatile acid formed by the oxidation of the aldehyde is propionic acid. These reactions show that a volatile aldehyde is formed during the growth of *Fusarium cubense* on Uschinsky's solution and that this aldehyde may be propionic aldehyde, although the amount of the aldehyde which was obtained was too small to make absolutely certain its identification as propionic aldehyde. The solution remaining in the flask after the fractional distillation of the volatile aldehydes gave no reactions for aldehydes either of the aliphatic or aromatic series.

A badly infected banana stalk received from Trinidad by the Laboratory of Plant Pathology was examined for the presence of aldehydes. The stalk was finely chopped and pressed in a fruit press, and the juice so obtained was examined by the method given above, but no aldehyde reactions were obtained.

Since propionic aldehyde is a very volatile compound it is possible that appreciable quantities were formed during the growth of the Fusarium and escaped from solution. The author had hoped to take up this question and also establish absolutely the identity of the aldehyde formed, but circumstances do not permit of this at present. The observations made seemed of sufficient interest to call to the attention of other workers on this subject. The author wishes to thank Dr. Caroline Rumbold and Miss Florence Hedges for their kindness in growing the Fusarium and for furnishing the diseased and healthy banana stalks.

U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

DOES CRONARTIUM RIBICOLA WINTER ON THE CURRANT?

W. A. McCubbin

With One Figure in the Text

In the literature at the writer's disposal very little mention has been made of the over-wintering of *Cronartium ribicola* on the currant. In a few cases there has been a suspicion of over-wintering on this host but either the evidence was too meagre to be satisfying, or else other facts appeared later to explain the circumstances so that the general opinion at present favors the entire dependence of the currant stage on a yearly infection from the pine.

In some of the records, however, there are mentioned puzzling occurrences of the rust on currants, either at long distances from pines, or in circumstances otherwise so suspicious as to suggest that the fungus might have passed the winter on the currants themselves. Spaulding (5a) has mentioned, in connection with the distribution of the Peridermium stage that judging from analogy with *Cronartium Comptoniae*, the spores of which are similar in size and shape to those of *P. strobi*, it is probable that the latter would be blown only relatively short distances; but he records two cases in which no diseased pines were to be found near rusted currants. In another article the same author (5) lists a reference to an observation made by Nilsson in 1893, where rusted Ribes were found over three quarters of a mile from any pines.

Efforts have been made to settle the question experimentally, both by planting out badly rusted currants in a disease-free neighborhood after wintering, and by inoculation with over-wintered spores. So far as is known the latter method has given only negative results. The former method was employed by Stewart and Rankin (9) using five hundred rusted currant plants. None of these developed any rust during the succeeding summer, and the conclusion is drawn that the fungus rarely, if ever, over-winters on the currant. In 1914 Spaulding (6), in recording the negative results from similar experiments with two hundred plants, says, "The practical conclusion is that Ribes plants do not carry the fungus over the winter and that an outbreak of this disease on Ribes is to be attributed to the presence of neighboring white pines which have the blister rust."

In a former article (3) the writer has detailed some of the circumstances of the Ontario outbreak which engendered a suspicion that the rust in question might have passed the winter on currants, and in the work with this disease in 1916 additional evidence has appeared to strengthen this suspicion. The evidence at present available can not be considered sufficient to establish the point beyond question, but it is important enough to be worth careful consideration.

In the discussion following several points which have a bearing on the question are considered: (1) A hypothesis to account for the various phenomena observed. (2) Agreement of this hypothesis with known conditions in other rusts of a similar nature. (3) The general and irregular appearance of the currant stage over large areas in which there is reason to believe no pine infections are responsible for the disease. (4) Special cases, where rust has occurred on currants which are far distant from any possible source of infection. (5) The occurrence of currant rust in one instance on two out of four plants in a plantation in which the same four plants, and these only, were badly diseased in the preceding year. (6) The occurrence of a case of currant rust on plants set out in a rust-free district in order to test over-wintering.

1. HYPOTHESIS TO ACCOUNT FOR VARIOUS PHENOMENA OBSERVED

On account of the difficulty arising from the loss of all the currant leaves in the fall, thus separating the fungus from its host, no satisfactory hypothesis has been brought forward to account for suspected cases of hibernation. Spaulding (1911) has suggested the possibility of this hibernation, and has put forward the idea of a hibernating mycelium, justifying it by reference to observed cases where pustules of Puccinia occurred on currant shoots. The presence of *Cronartium ribicola* in such suspicious locations does not seem to have been established, although the same author (5a) records the finding of the telial stage of *Cronartium ribicola* on petioles and stipules of Ribes.

Judging from observations made during the last two years, the only hypothesis which seems to account for all the observed phenomena in connection with supposed cases of wintering over, is the hibernation of the mycelium in infected buds.

A feature of the disease previously mentioned by the writer (3) has an important bearing on this phase of the question: Early and complete defoliation by the rust, followed by a secondary production of leaves, due to the premature opening of winter buds. It has often been observed that such secondary leaves are also rusted, even when they are only partially opened, and considering that the incubation period of the fungus

is from ten days to two weeks or more, it must be evident that infection can take place very close to the bud stage, perhaps as soon as the bud scales are parted enough to expose the young leaves within. Now in the case of a shoot producing secondary leaves in this way, the terminal bud opens first and makes most growth; the one next below it opens to a lesser extent; and those farther down exhibit diminishing degrees of activity, until towards the base of the shoot buds are found which still remain in a quite dormant condition. It is true that leaves produced in this way from buds opening late in the fall are killed by the first severe frost, and often several of the uppermost buds also perish, but some of these forced buds, without doubt, are able to survive the winter.

The fact that each shoot producing secondary leaves has its buds arranged in a series extending from the fully opened condition to the dormant state, shows that there is ample opportunity for such a favorable combination of circumstances to occur, while the actual presence of the rust on very young leaves is evidence of the capability of the fungus to infect at this season of the year. The only step that need be taken outside the realm of fact concerns the assumption that an infection can take place early enough in the development of a bud to still leave it capable of passing the winter.

The field conditions demanded by the above hypothesis are quite adequate for the purpose. The early defoliation mentioned is general in some plantations, and in a large percentage of others a smaller or larger area of plants lose their leaves in midsummer on account of rust starting from one center. The total number of such cases where secondary foliage has been produced is many times the number of suspected cases of wintering-over, so that even allowing for a lack of infection in some instances, dying out of the mycelium in winter, and so forth, there still remain several times the number of plantations or parts of plantations required to explain the observed outbreaks of the rust.

In this connection it may be noted that the climatic conditions of the Niagara Peninsula are extremely mild for the latitude; the autumn is long and open: in the last two years roses have been in bloom in November; the temperature in winter rarely falls below —12°F., and there is an early start of growth in the spring. Under such conditions the persistence of the rust on currant foliage until late in the fall, and its ready occurrence on the secondary foliage, is not a matter of wonder. In addition the buds are advanced in this mild fall weather far beyond the stage at which they usually go into the winter in other localities. Perhaps the less severe winters might also permit the mycelium to remain alive in infected tissues in a manner that would be impossible in colder localities.

2. AGREEMENT OF HYPOTHESIS WITH ANALOGOUS CASES

The view that the mycelium of the fungus might successfully pass the winter in currant tissue is open to no *a priori* objection. What is the constant habit of the fungus in the pine might well become a temporary or occasional happening on the other host. In this connection it is scarcely necessary to point out the known habits of other rusts under like circumstances, but the recent work of Meinecke (4) on *Peridermium harknessii* has a peculiar interest here. In summarizing this work he says, "In California *Peridermium harknessii* and *Cronartium Quercuum* are to a high degree independent of each other;" and again, "*Cronartium Quercuum* over-winters on *Quercus agrifolia;* new urediniospores form in spring around the old, dead sori on old, living leaves, and infect the young leaves." He believes that since the crop of new spores is formed around the old dead spots, that therefore the mycelium must over-winter in the leaf tissue. The same over-wintering of the mycelium has been found by Mains (2) to take place in *Coleosporium Solidaginis*, the perfect form of *Peridermium acicolum*. In this case the pustules of the fungus were found to arise in spring in the rosette leaves of the Solidago host, in which the mycelium had apparently over-wintered. He was able to prove this point by sectioning the leaves, and finding therein the rust hyphae in limited areas.

3 AND 4. APPEARANCE OF CURRANT STAGE WHERE PINE INFECTIONS DO NOT OCCUR

Owing to the suspicions that arose in 1915 concerning the possibility of over-wintering on the currant, a careful inspection was planned for 1916, in order to determine whether cases of early infection could be found, which were either so far away from pines as to preclude the possibility of pine infection, or which were close only to pines of small size, whose freedom from disease could be absolutely established. In general there are such numbers of large pines scattered over the whole of the Niagara Peninsula that, even should cases suspicious of over-wintering be found to occur on currants, the nearness of the other host would render these cases valueless from this point of view. In two areas, however, the pines were so few in number that an early appearance of the currant stage in them would be difficult to explain on the ground of pine infection.

One of these areas comprises that part of Grantham and Niagara townships included within the dotted line on the map (fig. 1), which it will be noted also records the position of all pine and currant plantations. In 1915 the rust in this area did not start from a particular center or centers

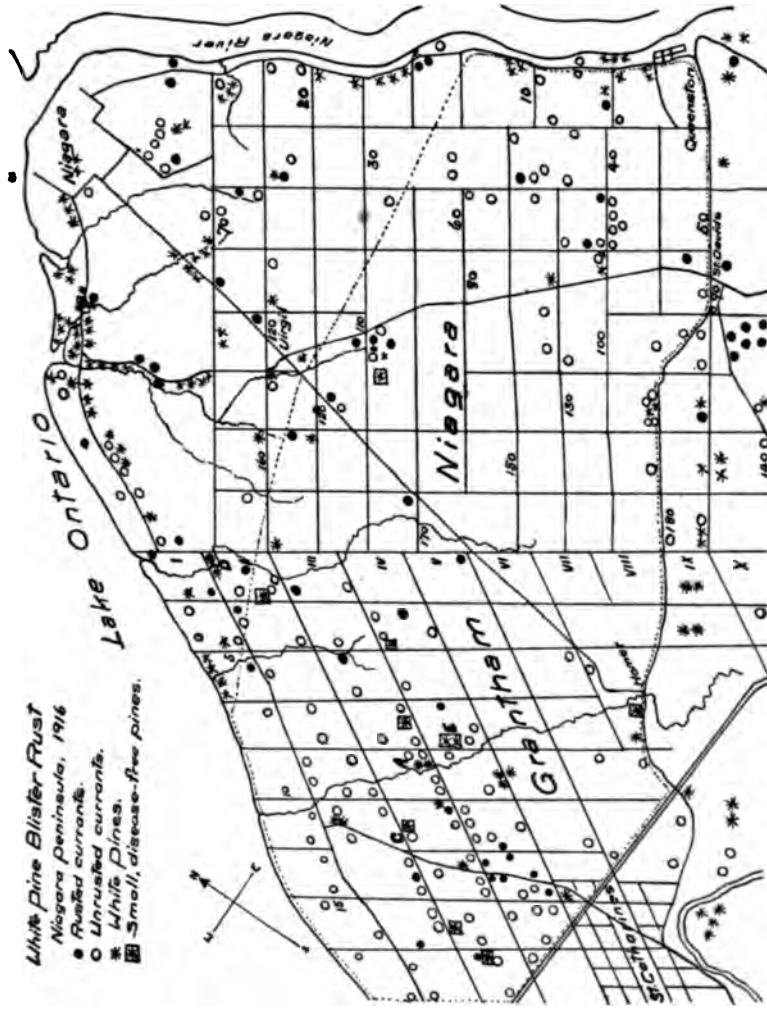

FIG. 1. MAP OF THE BLISTER RUST SURVEY IN GRANTHAM AND NIAGARA TOWNSHIPS, JUNE AND JULY, 1916

All pine groves and single trees, as well as all black currant plantations are recorded. Scale, two miles to the inch.

and spread outward therefrom, but it appeared simultaneously and irregularly over the whole district, totally without reference to the few pines to be found there. In 1916 the very same irregular occurrence was noted. Except in the Secord case hereinafter mentioned, there were nowhere any signs which would indicate pine infection; the infection areas in the plantations of currants were usually few, often only one or two; in no case was early infection general over a number of adjacent plantations, or even plentiful in one; and in most cases the only pines that could be suspected were more or less surrounded by disease-free currants.

Of the twenty-nine cases of currant rust (plantations) found in the area indicated, sixteen were so situated as to be regarded with strong suspicion. Eight of the sixteen are over a mile distant from any pine which could possibly be a source of infection, and in all the twenty-nine, except in the Secord case noted below, the rust started without any apparent reference to the pines in the neighborhood. Eleven of the twenty-nine cases originated on either one or two bushes in a plantation, and in all of these eleven the primary source could still be distinguished on the early leaves of the shoot. In sixteen other cases of the twenty-nine the origin from a similar small beginning was evident, but owing to lack of certainty in these cases they are not included as evidence.

Three of the cases occurring in this area deserve special mention on account of their typical character, and because of the strong evidence they bring on the question.

No. 1, Lot 17, Con. 3, Grantham. In this plantation there were present on July 6, two badly diseased bushes, on both of which the early infection had started on the second leaf of the shoot and had spread from there over the rest of the bush and to the adjoining bushes only. There are about twenty young pines four hundred feet from these currants, but they have been examined several times in 1915 and in 1916, and are all entirely free from the disease. Aside from these the nearest pines are over a mile distant with disease-free currants intervening.

No. 2, Lot 5, Con. 3, Grantham. Here are four old black-currant plants in a somewhat neglected garden. On July 6 two leaves on one of them had very old infection spots, while around these on the bush secondary uredino-pustules were present. The other bushes were absolutely free from the rust. As may be seen by reference to the map, there are three lots of pines southeast of this place, and one northeast. All these are young pines and are free from any signs of the blister rust. This disposes of all the white pines for nearly two miles in every direction except one forty-years-old tree in Lot 164, Niagara Tp., which is one and a fourth miles distant.

No. 3, Lot 36, Niagara Tp. The map shows the infected plantation to be over a mile from any pines whatever. It is moreover in the center of a currant area of which it alone is rusted. On July 15, the disease was found at one end of this plantation and the outbreak was traceable to several very old spots on early leaves.

In the second of the two areas mentioned, which includes the townships of Willoughby, Crowland and Bertie, in Welland County, the conditions are exactly the same as have just been outlined for the Grantham-Niagara district. The 1916 infection is irregularly scattered over the whole territory without any reference to the pines. Out of 185 currant plantations examined here seventeen were found to be diseased, of which fourteen were suspected to be cases of wintering over. In four of the cases the currant rust was found from one to two miles from the nearest pines, and even then these pines were not under suspicion, being either themselves far from a source of infection from currants, or else having disease-free currants near them.

Although the evidence adduced has been confined to the rust outbreaks in these limited and favorable districts, it must be understood that the same conditions prevail in other parts of the peninsula, and it is only the near presence of so many pines in all these districts which precludes adding a large number of other cases of a like suspicious nature. Unless either the aeciospores or the uredinospores are carried by the wind to much greater distances than we are accustomed to think, or than our limited experiences would indicate, the occurrence of currant rust in this area in 1916 is very puzzling on any other hypothesis than that of wintering over on the currant.

In addition to the above there is another line of evidence which has a direct bearing on the problem of the origin of the yearly rust outbreak in the Grantham-Niagara area already mentioned. This evidence is derived from careful examinations made of cases in the district where young pines and black currants are growing in close proximity, and of these the most outstanding instance was on Lot 13, Con. 3, Grantham, which for convenience of reference is called the Secord case.

On the Secord farm there was a row of sixteen young white pines planted along the western boundary. These pines were obtained from a native wood-lot in the neighborhood, and were planted out in 1910. Running from a lane in the middle of the farm to this row of pines are 175 large black currant bushes in five rows. On the eastern side of the lane, and about ninety yards farther north, is a small nursery plot of evergreens, among which were included about 150 young white pines, planted in 1912, and obtained from the Provincial Forestry plantation in Norfolk County, where they had been grown from native seed.

Although the currants on this farm were badly rusted in 1914 there was no sign of the disease on either lot of pines that year. In the season of 1915 the pines were all examined four times (May 5, May 14, June 14, August 9), but nothing remotely resembling the blister rust was found. The rust appeared again on the currants in the course of the summer, but was late in making its appearance, and possibly came in from elsewhere. Another inspection of the young pines was made very early in the spring of 1916, with the same result as before: no sign of the disease could be seen.

Owing to the close association of the two hosts, and the known occurrence of the currant stage for at least the two preceding years, special attention was judged to be necessary in this case, and accordingly a further inspection was made on June 6 and 7. On this occasion there were found a large number of discolored swellings which were undoubtedly the early

TABLE I

Total number of white pine blister rust swellings found June 6, 1916, in the Secord nursery and fence row and the age of growth on which they occurred

YEAR OF GROWTH	NURSERY	FENCE ROW	TOTAL	PERCENTAGE
1915	0	1[1]	1	0.5
1914	43	84[2]	127	71.7
1913	39	10	49	27.7
1912	0	0	0	0
Earlier	0	0	0	0

[1] Somewhat doubtful. A swelling below the end of a broken-off terminal branch.

[2] One of these produced the blisters noted above. It was located at the upper end of the internode developed in 1914.

stages of the blister rust, and which had developed so as to be visible since the former visit. Only one case of the blister stage was found: A small twig near the ground bore five small blisters. These had already shed their spores, but were still readily recognizable by the slit-like openings, remnants of the peridium, and by a few remaining spores. The pines were minutely examined over every part of the stems, branches and twigs, and all the swellings collected for study, after which the trees were destroyed. The collection of swellings was then carefully gone over in the laboratory, and a record was made of the age of the growth on which they occurred. Their position on the tree is given in tabular form below.

Since the two hosts are here so closely associated that the currant stage could hardly be present without causing some infection on the pine, we are enabled to form from this table some conclusion regarding the date of the first appearance of the disease in this particular locality. As no

pine infections have been found on any growth prior to and including 1912 it seems certain that there was no currant rust here before 1913 at least. It is, of course, possible that sporidia of 1913 might have infected twigs of 1911 or 1912, but if so it is hard to believe that all such infections are still dormant when later pine infections have developed regularly and vigorously on the same trees. This seems to hint at the absence of the currant rust here in 1913, but this part of the subject will be considered later.

Since the currants have been known to be badly rusted in 1914, 1915, and 1916, it is only reasonable to suppose that plentiful infections have taken place in each of these years on pines so favorably situated, a point which is well borne out by the presence of such large numbers of them on the wood of 1914. These must obviously have arisen after the wood was formed, and are therefore referable to the rust of 1914 or 1915. The above table, however, shows an entire absence of infections on 1915 twigs (save for one very doubtful and abnormal case), and since no swellings or other indications of the disease were seen here in 1915, although the pines were certainly subject to infection in 1914, it seems fair to conclude that during the season after infection the fungus produces in the twigs no symptoms of a visible nature.

If in addition to this year of dormancy it is assumed that the swellings formed in 1916 will reach the blister stage in 1917, it is probable that the disease in pine limbs follows a four-year cycle, which may be thus summarized: first year, infection in late summer or fall; second year, dormant period; third year, swelling and discoloration, with possibly pycnospores; fourth year, production of aecia.

This rule of development is not to be regarded as invariable. In some cases the dormant period may be extended considerably, and on the other hand cases are known where blisters have been formed in the second year after infection instead of the third. Under ordinary circumstances, however, this cycle is perhaps generally followed.

We may therefore consider the swellings included in the above table as having originated from infections of either 1914, or both 1913 and 1914. Since no record exists of the presence or absence of the rust here before 1914, we are unable to state that these pines were not exposed to infection in 1913. But if they were so exposed and infections resulted, we are compelled to give an explanation of the sudden development into the swelling stage of infections of two seasons, simultaneously in the spring of 1916. If currant rust was present in 1913 it is obvious that every one of a large number of infections made in that year must have remained dormant for two years, while on the self-same trees a still larger number starting in 1914 reached the same stage concurrently, after only one

year's dormancy. One might imagine that adverse weather conditions might bring about such a peculiar varation in the dormant period, but it must be noted that during the spring and summer of 1915 there were collected at Fonthill, some fifteen miles away, about two hundred pine infections, in all of which the swelling stage in both old and incipient cankers was abundantly active. Aside from this one would hardly expect *all* these cankers to be affected by weather conditions; some of them might be retarded but others more favorably situated would have followed the usual course, and would have appeared as swellings in 1915. It seems far more reasonable to regard all the swellings in the table as the result of a single year's infection, that of 1914. According to this view they have all followed the cycle indicated (with the exception noted), and are due to produce aecia in 1917. It would follow from this, that the currant rust could hardly have been present on these currants prior to 1914, the year it was first discovered here.

Since the Secord farm, (fig. 1, *A*), is very centrally situated in the township of Grantham and in the heart of a region thickly planted with currants, the absence of currant rust here is strongly indicative of its absence in the surrounding neighborhood. Additional evidence on this point is furnished by four other cases of a like nature in the same district, where pines of small size also grew side by side with black currants.

In the first of these, (fig. 1, *B*), a large currant field is 25 yards distant from a number of young native pines, on which no signs of the disease could be found in 1915, In October, 1916, there were found on fourteen of these pines thirty-eight cases of the blister rust swellings. Of these sixteen were on the growth of 1913 and twenty-two on that of 1914. None could be found on growth of 1915 and nothing was present on any wood prior to that of 1913.

In the second case (fig. 1, *C*) there were two young pines within six feet of the black currants. In 1916 these also developed swellings for the first time; of the five seen here two were on wood of 1913 and three on that of 1914.

In the third case (fig. 1, *D*), the few young pines were about fifty yards from a short row of black currants. The one case of pine infection which appeared here for the first time in 1916 was on a shoot developed in 1914.

The fourth case (fig. 1, *E*), disclosed only two pine infections on a dozen young trees which were within ten feet of a large but slightly affected black currant plantation. Both infections were on pine shoots of 1913 growth. There is some additional evidence here in the fact that this row of pines was transplanted to its present situation in the spring of 1914 from a hollow about four hundred yards from these currants. Since there were no other currants within a quarter of a mile of this hollow,

there is good reason to believe that the pines received their infection after they were transplanted, that is, in 1914.

In all the four cases just referred to the adjacent currants are known to have been diseased in 1914. Likewise the pines were in every case minutely examined in 1915, so that their freedom from disease until the spring of 1916 is well established. Since in every one of the blister cankers found in these four cases the disease has occurred only on wood of 1913 or 1914, and none have been visible till the spring of 1916, they agree in every way with the evidence obtained from the Secord case, and together with it give a very strong support to the view that the currant rust was not present in the Niagara-Grantham region before 1914.

The bearing of this conclusion on the question of wintering over is obvious. If the rust did not appear in this district until 1914, then according to the probable life-cycle of the fungus no pine infections could reach the blister stage and start new currant infection before 1917. It follows therefore that the currant rust of 1915 and 1916 in the area under consideration must be due either to spores carried for long distances by the wind or to a wintering of the fungus on the currants themselves. Since this area is about ten miles long by five miles wide, and is distant from the nearest area of infection (the Fonthill district) from ten to sixteen miles, any attempt to attribute all these rust outbreaks to wind-blown spores is full of difficulty. Aside from this there remains only the wintering of the fungus on the currants as an explanation.

Objection may be made to the above reasoning on the ground that in some instances aecia may be produced the second spring after infection instead of the third. It is probable that such cases are rare. The single instance of this sort that was met with in the Secord case was the only one out of 223 blister cankers collected in these two townships in 1916. The small branch on which it was found was protected by high grass, and had the puffed, swollen appearance which normally occurs when a small limb is stimulated by contact with the moist earth. It was evidently an abnormal case, and probably had undergone a forced development by reason of its peculiar conditions. The writer is of the opinion that such abnormalities would rarely occur in large trees, and as for the small pines, practically all of these in the two townships have been subjected to such a scrutiny that the possibility of their playing a part in the yearly outbreak is utterly out of the question. This is especially apparent when the extent of the early occurrence of the currant stage is taken into consideration along with the observed rate of spread from the infection started at the Secord farm by the abnormal blisters just mentioned. In this case the rust began about June 1, at the ends of the five rows of black currants. By July 6, it had progressed down the rows for a distance of

only two hundred feet, and two adjoining plantations distant two hundred and three hundred yards respectively, were still free from any sign of infection. By August 21, there was only a slight infection on these adjacent plantations. It is almost impossible to think that one small infection of this sort, or even a number of them, could have started such a widespread infection on currants as our survey disclosed.

Moreover, while an objection of this nature might be valid for the rust epidemic of 1916, it could hardly apply to that of 1915 which was of the same extent and in the same area. Assuming that the rust entered this district in 1914 any explanation of the succeeding year's outbreak on the basis of pine infection would require the production of the blister stage in the spring of 1915, from a blister canker started by infection during the previous autumn. Even admitting the possibility of such precocious development, the blisters formed in this way must either have been numerous and widespread, or the spores from one or a few of them must have been carried from five to ten miles. In the first case our careful examination of young pines must have disclosed some of them, at least; and in the second case the general and irregular occurrence of small outbreaks without any recognizable center of infection, together with the known behavior of the already mentioned outbreak of this nature on the Secord farm, are quite against any such explanation.

5. RECURRENCE ON SAME INDIVIDUAL

The hypothesis advanced above is capable of being tested to some extent by field observations. If the rust winters in buds forced into late growth by premature defoliation, then it should be possible to establish a connection between suspicious early outbreaks of the rust and the plants or small areas which were defoliated during the preceding summer; if these areas showing forced growth were marked in the fall and rust appeared in them in spring in a larger percentage of cases than in the ordinary parts of the field, the case for over-wintering would be very strong indeed. Such field work would necessarily have to be done in a district where the results would not be interfered with by the presence of too many pines.

Unfortunately no systematic effort has yet been made along this line. The small amount of evidence now in hand comes from three plantations which were marked as defoliated in 1915. Out of the three, two were subject to early outbreaks of the rust in 1916, while the third was free. In both of the two cases the infection was small and started from one or two centers within the defoliated portion. A perhaps more significant case came up in the field work of one of the inspectors. In one large

black currant plantation the inspector who covered the ground in 1915 reported only four bushes, adjacent in a row, as badly rusted, with but slight infection elsewhere on adjoining plants. These four bushes were seen at that time by the owner, and when the inspector of 1916 called here, the owner told him where to find these bushes, which were on the side of the plantation opposite a small tree. The only case of rust which could be found in this plantation was on one of the four plants mentioned. It is conceivable that spores blown from some other place might start an infection in this one spot, and there only, in two successive years, but it is so utterly improbable that one can scarcely avoid turning to the wintering-over hypothesis for an adequate explanation of the case.

6. RUST ON TRANSPLANTS IN A RUST-FREE DISTRICT

In a former article (3) mention has been made of a rust outbreak which occurred in a small plot of black currants set out in the spring of 1915 to test for hibernation. The one hundred currant bushes used were all badly rusted in 1914. They were divided into five lots, of which two were well sprayed with lime-sulphur, two were left unsprayed, and one was exposed to infection from rusted currant leaves wintered out-of-doors and suspended among the foliage in loose wire baskets. A locality was chosen for the experiment far away from any known rust area; this district had few pines and the freedom from rust of the few existing currants was ascertained during the fall of 1914.

The one case of rust which developed on these plants was on one of the sprayed plots. At the time of examination, October 18, it was still of very small extent, involving only one shoot of a single plant. There was a small original rust spot surrounded by about twenty others of more recent date.

Owing to the limited extent of the rust here so late in the season there was some hesitation in attributing it to a wintering of the fungus, and every other possible source of infection was given due consideration. The only one of these possible sources that had any degree of probability was the carrying of aeciospores to this place in an inspection visit made on May 24. At this date no currant rust had been met with but some inoculations with the aeciospores had been made in the laboratory on May 20. The writer did not do this work himself but was in the room at the time, and a few air-borne spores might have adhered to his clothing and have been thus carried to the field in question four days later. Improbable as this suggestion may seem, it is the only explanation on the basis of accidental infection which seems to be worth consideration.

On the other hand the wintering of the fungus on the currants themselves readily explains the case. The fact that the plants were well sprayed in spring is in perfect harmony with the hypothesis advanced. Even the late appearance and small spread of the infection, which would appear to be incompatible with wintering-over, need present no difficulty; it is well known that while *Puccinia graminis* may live independently of the barberry for indefinite periods, yet by undergoing its proper stage on this host its virulency on cereal hosts is much increased. It should not be too much to expect, then, that in the case of *Cronartium ribicola* the second successive seasonal generation on the currant should lack somewhat of the vigor it would possess after coming fresh from the pine. The results of the survey work in the Niagara Peninsula seem to hint at a confirmation of this view. A great many of the cases of currant rust observed here during the last two years have been in the type indicated: an old but very feeble rust center, which by the end of the summer had involved only a single bush, or at most a few adjacent bushes. Other explanations of this seeming lack of vigor are no doubt possible, but such a decadence is quite compatible with a hibernation hypothesis.

In concluding this discussion it is clearly recognized that the evidence submitted is inadequate to establish the point under consideration, but on the other hand it is considered that enough evidence has been adduced to warrant a strong suspicion of currant hibernation, and this suspicion holds even though the hypothesis tentatively put forward should prove to be untenable in the light of later investigation. In any case the evidence obtained serves to narrow the field of inquiry to a great extent; for the conditions outlined above are such that the question of hibernation clearly hinges on the distance aeciospores or uredinospores can be carried by the wind; if only for a mile or two, then wintering on the currant has almost certainly taken place in the area under consideration; if on the other hand the spores are borne eight or ten miles or farther, another explanation of the situation in this district becomes easily possible, although the question of hibernation is not even then altogether disposed of. In the absence of definite information concerning spore dispersal the question must remain open until a body of trustworthy evidence can be accumulated on this point, or until more direct evidence is available on other phases of the subject.

DOMINION FIELD LABORATORY OF PLANT PATHOLOGY
ST. CATHARINES, ONTARIO

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THE INJURIOUS EFFECTS OF TARVIA FUMES ON VEGETATION

A. H. Chivers

The following article contains a brief description of the destructive effect of tar smoke on plants which the writer had occasion to study during the summer of 1914, together with a brief account of experiments carried on since that time in the laboratory, and under controlled conditions.

The tar compound which was in use for building purposes, and which seriously affected the neighboring vegetation, is sold under the trade name, tarvia. For melting the compound a single kettle was set up about sixty feet distant from the nearest corner, and three hundred feet distant from the farthest corner, of a garden which covered an area roughly a hundred and eighty feet square, and which contained both ornamental and crop plants.

A strong and constant wind carried the fumes over the garden for about four hours on the afternoon of August 10 and throughout the forenoon of August 11, during which time the leaves and stems of the plants became coated with a substance of a greasy nature.

The subsequent destruction of the plants was very rapid. The leaves soon curled and shrivelled, dried out and fell. At least twenty species and many varieties were affected. Poppies, *P. somniferum* Linn., *P. rhoeas* Linn., *P. orientale* Linn., candytuft, *Iberis amara* Linn.; marigolds, *Calendula officinalis* Linn.; azaleas, *Azalea viscosa* Linn.; sunflowers, *Helianthus annuus* Linn.; strawberries, *Fragaria* in varieties, and squashes, *Cucurbita pepo* Linn., and *C. maxima* Duchesne, were killed. Paeonies, *Paeonia* in varieties, were killed to the surface of the soil. Roses, *Rosa* in varieties; honeysuckles, *Lonicera tartarica* in varieties; currants, *Ribes* in varieties; raspberries, black raspberries and blackberries, *Rubus* in varieties, were defoliated, and in some instances were killed. Potatoes, *Solanum tuberosum* Linn., which occupied the greater part of the garden, were stunted and the yield was greatly reduced. All perennials showed the effects of the injury in the following season's growth.

BRIEF SUMMARY OF LITERATURE

Observations on the effect of vapor and dust from tarred roads, and smoke from melting tar on neighboring vegetation, have led to extensive

investigation of many tar compounds, and it has been found that while these compounds vary widely, the smoke and fumes from these are in general injurious to plants.

Gatin[1] investigated different substances used for the surface treatment of roads and his results tended to show that many trees, shrubs, garden plants and flowers suffered injury from the fumes given off by the tar, and also from the dust arising from the treated roads. The injury seemed to be proportional to the distance from the road, the amount of phenol in the compound, and the isolation of the plants. The effect was shown in the fading of the leaves which were spotted and blackened. The cells were plasmolyzed and the chlorophyll disappeared. Marked differences in resistance to injury on the part of some plants were noted.

Gatin and Fluteaux[2] found as a result of studies on leaves and branches of catalpa and locust that plants which have been submitted during the season to the dust from tarred roads had become considerably modified in respect to anatomical structure.

Mirande[3] made a study of the influence of the tarring of roads on plants, and concluded that the injury was done by vapors given off in considerable abundance during dry, hot weather. He stated that if trees and ornamental plants in cities are to be preserved the use of tar on roads should be made with care. The same author[4] investigated the effects on plants of a number of commercial products such as Carbonyle, Carbolineum and Carboneine, all of which contained creosote, and a number of which were used as insecticides. They were more or less injurious, causing the destruction of the green cells. He urged care in their application.

Griffon,[5] as a result of laboratory work covering three seasons, confirmed the conclusions of Mirande regarding the injurious nature of gases given off from tar when used in coating roads. From extensive observations

[1] Gatin, C. L. The effects of tarring roads on the growth of trees in the Bois de Boulogn. Compt. Rend. Acad. Sci. Paris. **153**: 202–204. 1911.

—— The experimental reproduction of the injury to plants by the vapors and dust arising from tarred roads. Compt. Rend. Acad. Sci. Paris. **153**: 688–690. 1911.

—— The tarring of roads and its effect on the neighboring vegetation. Ann. Sci. Nat. Bot., ser. 9, **15**: 165–252. 1912.

[2] Gatin, C. L. and Fluteaux. Anatomical modifications produced on plants by dust from tarred roads. Compt. Rend. Acad. Sci. Paris. **153**: 1020–1021. 1911.

[3] Mirande, M. The effect of tarring roads on plants. Compt. Rend. Acad. Sci. Paris. **151**: 949–952. 1910.

[4] Mirande, M. The effect on plants of certain substances extracted from coal tar. Compt. Rend. Acad. Sci. Paris. **152**: 204–206. 1911.

[5] Griffon, E. The influence of tarring roads on neighboring vegetation. Compt. Rend. Acad. Sci. Paris. **151**: 1070–1073. 1910.

he concluded that the probable injury to vegetation in the open country would be small.

Claussen[6] exposed plants to vapors of several commercial tars, and found that the various kinds of tar sold for building highways differed widely as to their effects on plants. The nature and extent of the injury were closely related to the concentration, thus depending on volatility and temperature, and that species of plants differed widely as to their susceptibility to the vapors. Certain recommendations were made in respect to the proper handling of such products.

Gabnay[7] gave a brief account of the injury to trees by tar used on the trunks as protection against the ascent of caterpillars, which involved not only the cambium but also the sap wood, and extended beyond the limits of the tarred areas. The injury was ascribed to the exclusion of air and the action of acids and salts.

Ewert[8] investigated the injury to vegetation by smoke-borne products, and reported a peculiar lacquered appearance on the upper surface of leaves of a number of economic plants, frequent rolling and crumbling of the laminae, and discoloration over part or all of the surface. Fruits and garden produce in such neighborhoods showed the effects. Controlled experiments showed that injury depended not alone upon the amount of material present in the atmosphere, but also upon the heat, dryness and isolation.

A case most similar to the one under discussion is recorded by Moore[9] as having occurred at Woods Hole, Massachusetts, when a collection of valuable roses was seriously damaged by smoke which resulted from the burning of a tar and gravel roof in the vicinity of the garden. The effect of the smoke began to be noticeable during the third day of the fire, and was indicated not only externally by the falling of the leaves and the scarring and marking of the young and tender stems, but also internally where large areas of growing tissue died and the contents of the cells were shrunken and distorted, the green coloring matter having been completely disorganized. All plants were affected, some were killed outright, and others so weakened that they became much more susceptible to the attack of fungous diseases.

[6] Claussen, P. The influence of tar, particularly that of tarred streets upon vegetation. Arb. Kais. Biol. Anst. Land. u. Forstw. **8**: 493-514. 1913.

[7] Gabnay, F. von. The pathological action of tar on plants. Centbl. Gesam. Forstw. **39**: 497-504. 1913.

[8] Ewert, R. Injury to vegetation by coal tar and other vapors, and protection therefrom. Zeitschr. Pflanzenk. **24**: 257-273, 321-340. 1914.

[9] Moore, G. T. Roses vs. Railroads. Rhodora **5**: 93-96. 1903.

The very complete bibliography of McClelland[10] also should be consulted in this connection.

RESULTS OF EXPERIMENTS CONDUCTED IN THE LABORATORY

It was soon found that the injury to plants by tarvia could be duplicated easily in the laboratory, and experiments have been made for the purpose of determining whether or not what seemed to be facts at the time of the accident would appear under controlled conditions. For this work begonias, Begonia in varieties; ferns, Adiantum, Aspidium and Pteris; wandering jew, *Zebrina pendula* Schnizl. and *Commelina nudiflora* Linn.; and geraniums, Geranium in varieties, were used.

In a comparatively short time after the plants were placed in the path of the fumes, the same greasy covering of condensed volatile substances which collected on the garden plants began to appear over the plant surfaces. Plants three to four feet distant from the source of the fumes showed an appreciable covering in about three hours.

The symptoms of injury were found to vary appreciably with the species. Leaves of begonias showed a characteristic sinking of the upper epidermis, at first in small, isolated areas, which gave a peculiar pocked appearance to the leaves. The pock marks gradually became confluent, and the entire area lost chlorophyll and turned brown. In the youngest leaves the first symptoms appeared as yellow spots, three to six millimeters in diameter, which when examined, were found in each case to be an injured area immediately surrounding a multicellular gland. Older leaves turned yellow over their entire surfaces and fell from the stem.

Ferns treated with the fumes withered and dried as if subjected to extreme heat. Geraniums showed a tendency of spotting. In general, however, the lower and older leaves turned yellow, those of medium age turned dark brown over the entire surface, while the youngest and only partially unfolded ones showed dark brown zones on their margins.

Experiments were performed with the purpose of determining whether or not the injury was due to the interchange of gases through the stomata. Species of begonias were particularly desirable for these experiments, since stomata are found only on the under surfaces of the leaves. Plants with a single stem bearing about ten leaves were used. The stem was wound with cotton and then with waxed paper. Some leaves were left unprotected. For other leaves cork masks were cut to fit the upper and under sides, the center of the upper mask having been cut away until only a narrow rim remained. These masks were then pinned in place so that the leaves were entirely protected on the stomatal surfaces, but exposed

[10] McClelland, E. H. Bibliography of smoke and smoke prevention. Mellon Inst. Indus. Research. Bul. **2**: 1-164. 1913.

on the upper surfaces with the exception of a narrow margin. The plants were placed so that the exposed surfaces faced the fumes. In all cases the injury was as marked and of the same nature as in the unmasked leaves.

An experiment was tried of painting onto the surfaces of the leaves with a camel's hair brush the volatile matter which condensed on the surface of the glass above the emanating fumes. It made little difference whether it was applied to the upper or lower surface of the leaf. The affected areas showed the same symptoms as those treated with the fumes. The painted spots became brown and finally dried and dead.

It was assumed from the first that the injury in the garden was due entirely to the effect of fumes on above-ground parts. To confirm this assumption, however, the pots were either wrapped in several layers of paraffined paper, or coated with paraffin and their tops covered with waxed paper. With plants thus protected the results recorded above were obtained.

SUMMARY

The results may be summarized as follows:

1. The fumes from the compound known as tarvia are highly injurious to vegetation.

2. Leaves whose surfaces were painted with the oily matter which collected on a cool glass plate over the emanating fumes showed the same symptoms of injury as did those treated with the fumes. This, together with other evidence, indicated that the injury was due in large part at least to the constituents of the volatile substances which condensed in the form of an oily coating on the surfaces of the plants.

3. Plants with no stomata on the upper surfaces of their leaves were protected in respect to all other surfaces, and so placed that only the upper leaf surface was subjected to the fumes. Such plants showed injury of exactly the same nature as did those with unprotected stomatal surfaces. This indicated that the injury did not involve, to any extent at least, the passage of gases through stomata.

4. In a sufficient number of experiments the soil and under-ground structures were protected from the fumes, showing that the injury was due to the action of the fumes on aerial parts.

5. The injury varied with the distance from the escaping fumes, the temperature of the melting tar, the age of the plant structures, and the species used.

DARTMOUTH COLLEGE,
HANOVER, N. H.

SOUR ROT OF LEMON IN CALIFORNIA*

CLAYTON O. SMITH

WITH TWO FIGURES IN THE TEXT

The fungus causing the decay described in this paper was first isolated some years ago from lemons which were originally infected with the brown rot fungus *Pythiacystis citrophthora*. Artificial inoculations made at that time on green lemons with a pure culture of the organism gave negative results, and no further attention was given to it until in 1915. The method of development, morphology and general characteristics of the fungus, noted at that time, were similar to those described in the present study. The decay has been found in many of the lemon packing houses of California and probably occurs more or less in all of them. The fungus is not known to have caused serious losses until the summer of 1915, when the unusually large crop of lemons made it necessary to hold large amounts of fruit in storage for a longer period of time than is customary, during which time considerable loss occurred in the packing houses and in transportation. It seemed to be especially infectious with fruit picked in the spring, being most commonly reported in May fruit. The fungus has also been found causing a rot of Valencia oranges in transit.

Several popular terms have been applied to this decay such as sour rot, slimy rot, watery rot. These terms are descriptive of different stages of the decay. The peculiar sour odor is so constant a characteristic and one so distinct from those of other decays of citrus fruits that the name sour rot is suggested for this decay.

Sour rot is a soft decay, during storage, of citrus fruit, especially of lemons. The tissue when infected quickly softens, but for some time may retain nearly its normal shape. It, however, changes to a straw color[1], later collapses, becoming more or less slimy with age, and at last is almost completely changed into a watery mass, which in the packing house often drips down into the lower fruit of the stacks. Because of these characteristics the grading and sorting of the fruit is very disagreeable. The softened areas of the fruit do not at first show any noticeable aerial my-

* Paper No. 38, Citrus Experiment Station, University of California, Riverside, Calif.

[1] Dauthenay, Henri. Repetoire de Couleurs, p. 31, No. 3.

celial growth, but later a short white (never glaucus), flaky (never very cottony), growth occurs and forms a thin, compact, often somewhat wrinkled, mycelial layer over the affected tissue. The fungus is infectious, readily spreading by contact to sound fruit in a way very similar to that of several other fungi causing rots of lemon fruits.

A microscopic study of the affected tissue, as well as of pure cultures, shows numerous spores of variable lengths. These spores frequently are flattened at the ends suggesting at once that they had a chain-like arrangement in growth. An examination of cultures of the fungus in petri dishes shows considerable aerial mycelium which is closely septate,

FIG. 1. SOUR ROT ON EUREKA LEMONS

The photograph shows natural infection as it is sometimes found in storage boxes in lemon-packing houses. The shrinkage in the part of the box having the rot is to be observed.

the individual segments being spore-like in appearance, and when placed in water readily separate as individual spores. The chains of aerial spores are simple or branched. The submerged mycelium is closely septate, especially when old. The individual segments readily separate from each other in water and function as spores. The spores measure 8–20 x 5–8 μ. They are oval to oblong, have obtuse often nearly square corners. When grown on certain media, as citrus fruits, oil globules and a granular substance are formed. The mycelium which measures 6–7 μ in diameter may when grown on a rich substratum like citrus fruits also contain granular matter and also possibly oil drops.

The fungus causing the sour rot is probably identical with the one origi-

nally described by Ferraris[2] as *Oidium citri-aurantii*. Saccardo and Sydow later transferred the fungus to the genus Oospora. Cultures of the sour rot fungus were submitted to Professor David R. Sumstine, Peabody High School, Pittsburgh, Pennsylvania, for identification, and he regards this species as belonging to the genus Oosporoidea,[3] a group of fungi that is now separated by some systematists from Oospora because of the slight differentiation between the mycelium and the sporophores. The fungus should now probably be called *Oosporoidea citri-aurantii* (Ferraris), but for the present will be designated as Oospora. The aerial mycelium of the sour rot readily separates into spores when mounted in water for examination, as does also the mycelium growing on the substratum. Sporophores and chains of spores are with difficulty distinguished from the mycelium.

Ferraris[4] found from his study and inoculations, that the fungus *Oospora citri-aurantii* caused an infectious soft decay of oranges. The individual points of infection increased in size and coalesced. A strong odor of fermented juice and a disagreeable taste of the fruit accompanied the decay. No aerial mycelial growth was at first visible, but under favorable conditions, a short very white, wrinkled mycelium developed, forming in contact with the substratum a gelatinous layer. The mycelium has a constant diameter of about 7 μ being described as being perfectly yellow and granular when growing in the orange tissue. The color of the mycelium of the sour-rot fungus as observed under the microscope when taken from artificially inoculated oranges, shows a slightly yellowish color but could hardly be said to be perfectly yellow. The size of the conidia as given agrees very closely with that of the sour-rot fungus. Ferraris recorded the size of cylindrical conidia as 13.5–19 x 7–7.5 μ; oval conidia 9–12 x 7.5 μ; spherical conidia about 12 μ.

Ferraris refers to certain other closely related fungi, causing rots of citrus fruits. Among these are *Oidium fasciculata* Berk., probably synonymous with *Oospora fasciculata* Sacc. et Vogl. and *Acrosporium fasciculatum* Grev., which is said to occur in decaying citrus fruits in Belgium, Great Britain, Italy and North America. This fungus differs from *Oospora citri-aurantii* in that the mycelium is at first white but changes to glaucus with age. *Oidium tigitaninum* was described[5] from California as a pow-

[2] Ferraris, T. Di un nuovi ifomicete parassita nei frutti di arancio. Malpighia **13**:. 1900.

[3] Sumstine, D. R. Studies in North American Hyphomycetes. The tribe Oosporeae. Mycol. **5**: 45–61. 1913.

[4] Ferraris, T. Loc. cit.

[5] Carter, C. M. A powdery mildew of Citrus. Phytopath. 5: 193–196. 1915.

dery mildew of Dancy tangerine. It differs in size, in shape of spores and in other morphological characters from the fungus under consideration.

Oospora citri-aurantii is closely related to *Oospora lactis* morphologically, a fact fully recognized by Ferraris, who found similarity in conidia

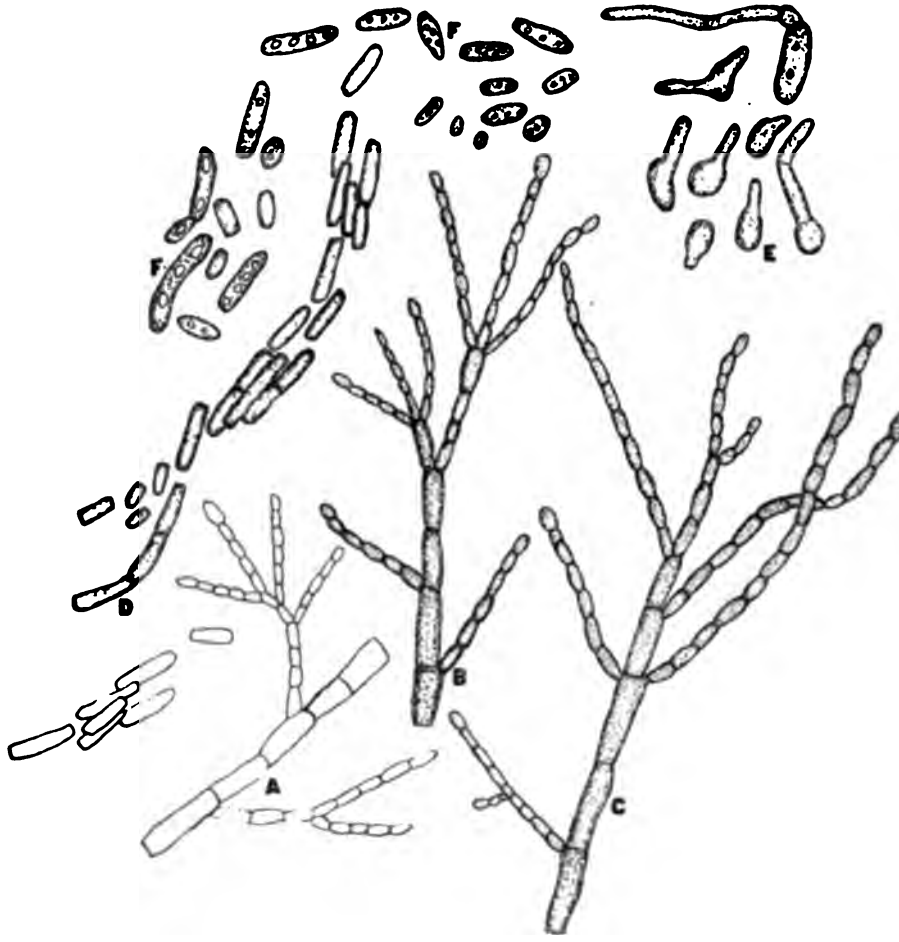

FIG. 2. SPORES AND MYCELIUM OF OOSPORA CITRI-AURANTII

A, *B*, *C*, aerial mycelium showing chains of conidia. *D*, mycelium separating into cells that function as spores. *E*, conidia, showing various stages in germination *F*, old conidia showing oil globules.

germination, hyphal branching, and the manner in which the conidial chains are formed. He also mentions the enormous number of conidia which these two fungi are able to produce, especially when the mycelium

itself breaks up into spores. Certain differences have, however, been found between these two fungi.

Oospora lactis is probably a composite species into which a number of closely allied fungi have been placed by systematists. A culture of *Oospora lactis*, with which the sour-rot fungus was compared, was furnished by Mrs. Flora W. Patterson. This culture was isolated from oysters. A culture isolated from the same source was also received from Dr. Charles Thom.

Artificial inoculations with *Oospora lactis* from these two cultures and with the sour-rot fungus on citrus fruits showed that *Oospora citri-aurantii* is pathogenic, causing the fruit to begin to decay within a few days. *Oospora lactis* at first produces some mycelial growth on the injured tissues, but the mycelium does not appear to be able to attack the tissue adjacent to the injury and no actual decay takes place. These experiments were performed several different times in moist chambers on Eureka lemon. Navel orange and Dancy tangerine. No infection took place, although duplicate experiments on the same kinds of fruit with the sour-rot fungus gave positive results.

The reaction of litmus milk with the two fungi differs. The sour-rot organism caused no change in the reaction and probably made but slight growth. *Oospora lactis* showed an acid reaction and clearing of medium without separation of the casein.

The spores of the sour-rot fungus appear more regular in size, and more cylindrical than those of *Oospora lactis*.

Artificial inoculations were made by puncture with cultures of *Oospora citri-aurantii* on the following citrus fruits in moist chamber: lemons, oranges, grapefruit and tangerine. Positive results were secured in moist chamber, the rot beginning to show in forty-eight hours. The ripe or nearly mature fruit is more readily infected. Failure to infect the green fruit of lemons has frequently occurred. Inoculations on the twigs of a Eureka lemon gave negative results. Lemons showing the initial stages of the brown-rot fungus, *Pythiacystis citrophthora*, were atomized with a suspension of spores of the sour-rot fungus. Infection took place quickly in the brown rot areas and continued to increase as the former decay advanced. Eventually the surface of the lemons was coated with the sour-rot fungus. Sound fruit when inoculated with an atomized suspension of spores or when soaked for twenty-four hours in spore-laden water were but rarely infected and then probably only in some superficial injury. Infection of lemons with the sour-rot fungus evidently only takes place through some injury or from contact with infected fruit.

WHITTIER, CALIFORNIA

A DISEASE OF PECAN CATKINS

B. B. Higgins

With Two Figures in the Text

During the latter part of April, 1916, the writer's attention was called to an abnormality of the catkins (staminate) of pecans, *Carya illinoensis*, on the Experiment Station plats. Some, or in many cases, all of the flowers of a catkin were slightly distorted and of a paler green hue. The stamens and inner surface of the subtending bract were covered with a white substance which at first glance gave the impression of white fly, but which on examination was found to be the white spore-cluster and basidia of a fungus belonging to the genus Microstroma. A little later when the pollen was being shed the contrast between healthy and diseased catkins was made more conspicuous by the failure of infested anthers to dehisce.

Sections of diseased anthers showed that, while the tissues in direct contact with the mycelium were not killed outright, the pollen grains were mostly degenerate, empty, and often collapsed shells. The mycelium is entirely intercellular, often forming thick mats which wedge the host cells apart and cause the slight distortion of the diseased parts. These mycelial mats become especially prominent at points near the surface where the large basal stroma of the fruit-body is formed in the loose subepidermal parenchyma. From this structure the club-shaped basidia push through the epidermis forming a small but compact hymenium above the surface of the host tissue. The individual threads of the intercellular mycelium and also of the stromata are extremely small and difficult to distinguish as such.

Apparently no toxic substances or injurious enzymes are secreted by the fungus, since the protoplasts and nuclei in the infested tissue retain nearly normal appearance. The changes in cells entirely isolated by the mycelial mats indicate starvation rather than toxemia. The pollen grains present similar evidences of starvation. The vacuole gradually enlarges and the protoplasmic layer becomes thinner until it disappears entirely leaving the empty pollen-cell walls which collapse or retain their original shape according to their degree of maturity.

IDENTITY OF THE PARASITE

Of the four species of Microstroma mentioned in Saccardo's *Sylloge Fungorum*, *M. album* (Desm.) Sacc. occurs on leaves of oak, *M. Cycadis*

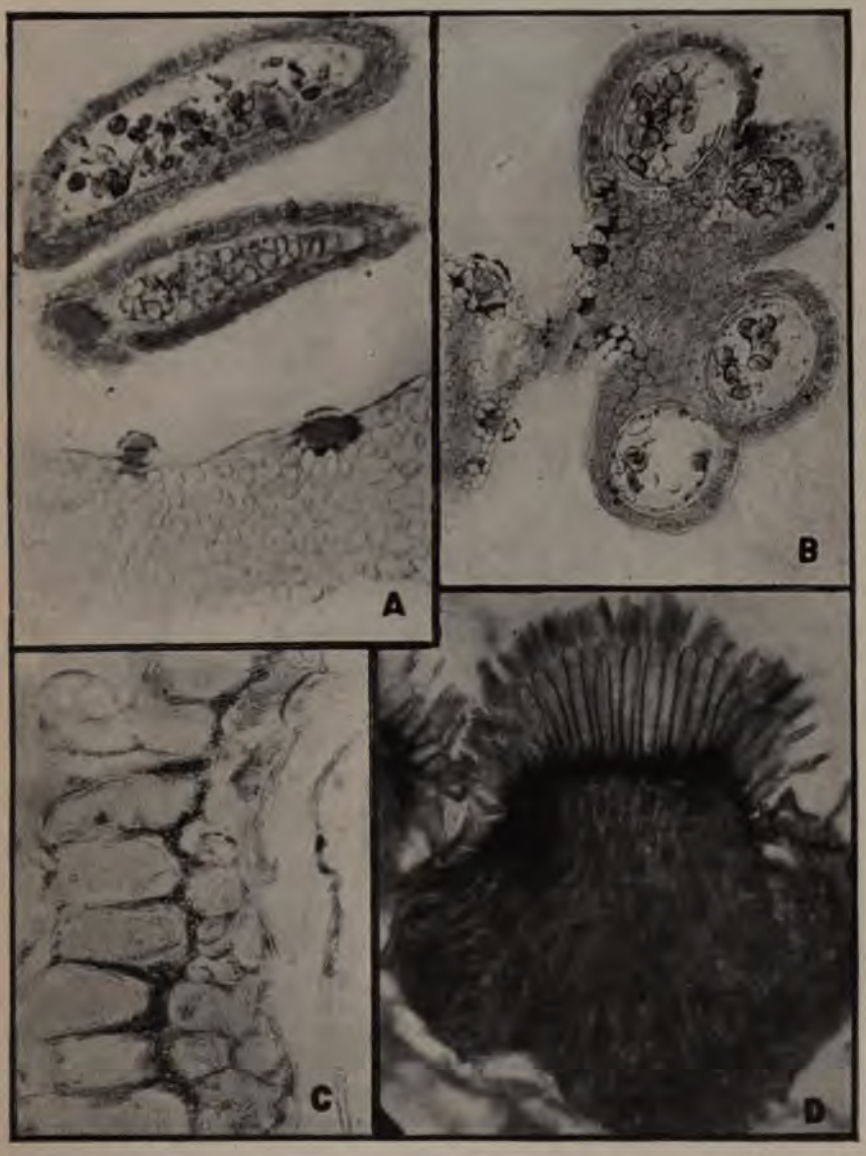

FIG. 1. MICROSTROMA JUGLANDIS VAR. ROBUSTUM ON CARYA ILLINOENSIS

A, longitudinal section of diseased stamen and subtending bract, showing degenerate pollen and also fruiting bodies and mycelial mats of fungus. × 75; *B*, cross-section of diseased stamen and bract. × 70; *D*, fruiting body of fungus from infected bract. × 500; *C*, mycelium between cells of pollen-sac. × 500.

Allesch. on leaves of *Cycas revoluta*, *M. americanum* Pammel & Hume on leaves of *Cnicus americanus*, and *M. Juglandis* (Bering) Sacc. on leaves of Juglans and Carya. Since *M. Juglandis* was abundant early in the spring on leaves of hickories, it was at once suspected that the fungus on pecan catkins was identical with this species. Comparative measurements of the various structures of the fungus from the two hosts showed however some very marked differences. The spores from pecan catkins are cylindrical, 9 to 14 by 5μ, and those from hickory leaves are oval to

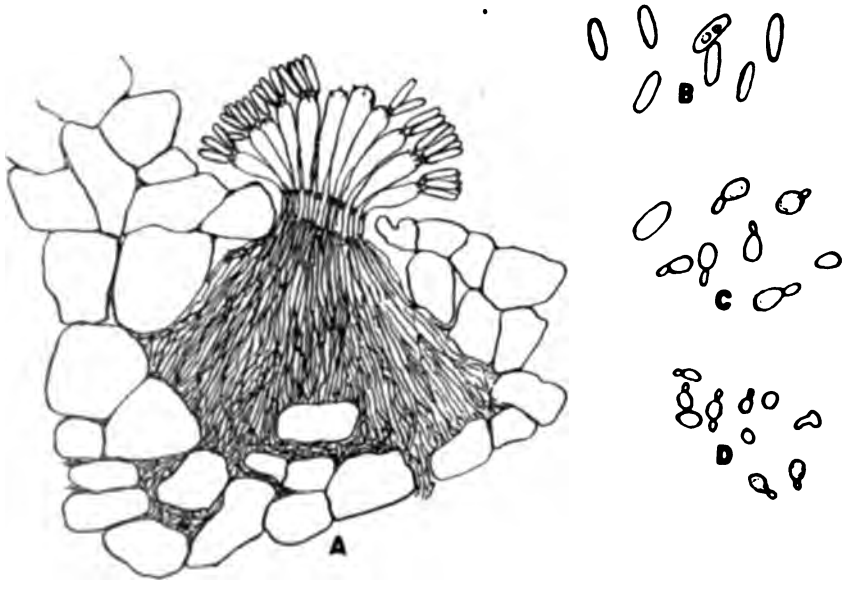

FIG. 2. CAMERA LUCIDA DRAWING OF MICROSTROMA JUGLANDIS VAR. ROBUSTUM

A, fruiting body showing basidia, sterigmata, and spores; *B*, fresh spores; *C*, yeast-like cells from eight-days-old culture on corn meal agar; *D*, cells from old culture on corn meal agar. All × 775.

oblong, 6 to 8 by 4.5μ. The basidia as well as the stromata from which they arise are much larger and the number of basidia from each stroma much greater on the pecan.

Cultures of both forms were obtained and compared on various media. The responses were very similar in each case. On all media so far tried, only a yeast-like growth is formed. The spores swell considerably and within a few hours begin forming new cells by a budding process, soon forming a small, circular white colony. At first short hyphal germ tubes were found occasionally, but they soon disappeared. As the budding process continues the daughter cells become smaller and oval or elliptical

to globose. The fungus is not long-lived in cultures, requiring frequent changes to new media.

Early in May direct and cross inoculations were tried with fresh spores from each host, but they all resulted in failure.

Diligent search for diseased catkins was made over several hickory trees the leaves of which were infested with Microstroma but none were found. Neither was any disease found on the leaves of pecan trees, although in some instances they were almost in contact with diseased hickory leaves.

Notwithstanding the apparent difference in tissues attacked, in size of spores, basidia and so forth, it still seems doubtful that the pecan fungus should be given specific rank. The more robust habit on the catkins may be due to the more abundant supply of food. Therefore for the present, it seems best to consider the fungus on pecan catkins as merely a robust variety of *Microstroma Juglandis* (Bereng.) Sacc. and to present the following diagnosis:

Microstroma Juglandis (Bereng.) Sacc. var. **robustum** n. var.

Host tissue pale, often slightly distorted; mycelium intercellular, forming more or less dense mats between the host cells; fruiting stromata oval to short conical, 60 to 100 by 55 to 150μ, composed of very slender interwoven threads; basidia club shaped, 13 to 30 by 5μ, bearing apically 6 to 8 spores on short sterigmata; spores hyaline, one-celled, cylindrical, rod-shaped, 9 to 14 by 3 to 5μ.

Hab. On stamens and staminate bracts of *Carya illinoensis*.

Microstroma Juglandis (Bereng.) Sacc. var. **robustum** n. var.

Stromatibus fructificantibus subepidermicis, ovatis vel brevo-conicis, 60 to 100 by 55 to 85μ; basidiis caespitosis, clavatis, 13 to 30 by 5μ., sex-v. octoporis; sporidiis hyalinis, cylindricis, 9 to 14 by 3 to 5μ.

ECONOMIC IMPORTANCE OF THE DISEASE

Since pollen is always produced in super-abundance by pecan trees the loss of a comparatively large amount is of little importance. Since however, on some trees fully one-third of the pollen was destroyed one can readily see how the disease may become serious in the near future. At present so little is known as to the life history of species of Microstroma that any suggestion as to control measures is almost valueless.

Observations in the Station orchard during the past spring indicated that few or no commercial varieties are entirely immune; but the attack was much more severe on some varieties than on others. Similar observations were also made in orchards around Albany, Georgia.

GEORGIA AGRICULTURAL EXPERIMENT STATION
EXPERIMENT, GEORGIA

SOME NEW OR LITTLE KNOWN HOSTS FOR WOOD-DESTROYING FUNGI

ARTHUR S. RHOADS

Despite the great extent to which wood-destroying fungi have been collected, but comparatively little attention has been paid to the host species on which they occur. One frequently finds in herbaria good collections the practical value of which is greatly reduced by being deficient in this respect. Within the last few years, however, increasing attention is being paid to the host species with the result that many new hosts have been established and many fungi which formerly were thought to be confined entirely to the wood of deciduous or coniferous trees are now known to occur on both.

In his own collecting work the writer always has been particularly interested in the hosts for wood-destroying fungi and frequently collects for host species alone. In looking over his lists recently a few species were noted, some of which apparently never have been reported. All but two of the collections cited here have been made by the writer himself or in conjunction with others, either in the states of Pennsylvania or New York. The following host species for wood-destroying fungi are believed to be new or at least little known.

Coriolus versicolor[1]

On dead trees, fallen trunks, and stumps of *Tsuga canadensis*[2] (Pa. and N. Y.).

On rustic fence rails of *Juniperus virginiana* (Pa.).

On fallen trunks and stump of *Abies balsamea* (N. Y.). This species was noted as a host by Dr. L. H. Pennington on two occasions in the Adirondack region.

Coriolus nigromarginatus

On a dead trunk of *Tsuga canadensis* associated with *Coriolus abietinus* (N. Y.).

Coriolus prolificans

On dead trunks of *Tsuga canadensis* (Pa. and N. Y.). Numerous sporophores occasionally are found either pure or associated with *Coriolus abietinus*. It pro-

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[1] The nomenclature for fungi used in this paper is that of William A. Murrill. [(Agaricales) Polyporaceae (pars). North Am. Fl. 9: 1-72. 1907; (Agaricales) Polyporaceae (concl.). North Am. Fl. 9: 73-131. 1908.]

[2] The nomenclature for trees used in this paper is that of George B. Sudworth. (Check list of the forest trees of the United States, their names and ranges. U. S. Dept. Agr., Div. Forestry Bul. 17: 144 p. 1898.)

duces a sap-rot in hemlock that is indistinguishable, macroscopically at least, from that caused by *C. abietinus*.

Coriolellus sepium

On rustic fence rails of *Juniperus virginiana* (Pa.).
On stump of *Tsuga canadensis* (N. Y.).

Tyromyces cæsius

On rustic fence rails of *Juniperus virginiana* (Pa.).

Bjerkandera adusta

On rustic fence rails of *Juniperus virginiana* (Pa.).
On stump of *Thuja occidentalis* (Pa.).

Porodisculus pendulis

On branches of fallen trunks of *Juglans cinerea* (N. Y.). This fungus usually is collected on wood of *Castanea dentata* but occurs commonly about Syracuse on butternut wood.

Polyporus Polyporus

On slash of *Tsuga canadensis* (Pa.).

Pycnoporus cinnabarinus

On a fallen sapling of *Tsuga canadensis* (Pa.).
On log of *Picea rubens* in corduroy road (N. Y.).

Hapalopilus gilvus

On a dead sapling of *Tsuga canadensis* (Pa.).

Ischnoderma fuliginosum

On dead trunk of *Pinus strobus* associated with *Coriolus abietinus* (N. Y.).

Elfvingia megaloma

On dead trees, fallen trunks, and stumps of *Tsuga canadensis* (Pa. and N. Y.).
On stump of *Abies balsamea* (N. Y.).

Ganoderma Tsugæ

On or in close contact with stump of *Pinus rigida* (Pa.).
On stump of *Picea excelsa* (Pa.).
On a much decayed stub of *Betula lutea* (N. Y.). A fine large specimen was collected on the latter host at Cranberry Lake, New York, and was fully as typical as those frequently found on hemlock trunks in that region.

Glœophyllum trabeum

On rustic fence rails of *Juniperus virginiana* (Pa.).

Glœophyllum hirsutum

On a soft maple log in a wharf at Oneida Lake, New York. Occasional sporophores were associated with its near relative, *Gloeophyllum trabeum*. The wood was either that of *Acer saccharinum* or *Acer rubrum*, but judging from the dominance of the silver maple in the lowlands of this region, it probably was the former species.

On stump and adjacent log of *Prunus avium* (Pa.). Near State College, Pa., sporophores were found at various times associated with *Gloeophyllum trabeum* on a stump and nearby log from the same tree.

On fallen trunk of *Betula lutea* (N. Y.). A collection of this plant was found in the herbarium of the New York State College of Forestry and, although no host was recorded, a few sporophores had ample bark attached to them to be positively certain that they grow on yellow birch.

Glœoporus conchoides

On an old sporophore of *Inonotus dryophilus* (Pa.). In making a collection of the former plant from a black oak log an old sporophore of *Inonotus dryophilus* was found on the log that also was well covered with sporophores of *Glœoporus conchoides* and seemed to be as good a host for this plant as the wood of the log.

THE NEW YORK STATE COLLEGE OF FORESTRY
AT SYRACUSE UNIVERSITY
SYRACUSE, NEW YORK

NOTES ON CRONARTIUM COMPTONIÆ III

PERLEY SPAULDING

In 1908 the writer collected a specimen of *Cronartium Comptoniæ* Arthur on a young tree of *Pinus rigida* at Burlington, Vermont. Since that time all available information concerning this fungus has been accumulated, and all the experimental work possible has been done. Three brief papers[1] have been published giving some of the more important facts which have been learned. It is proposed in the present paper to state very briefly some results secured in more recent investigations.

The pine hosts of *Cronartium Comptoniæ*, which have been previously reported by various writers, are *Pinus rigida* Mill., *P. sylvestris* L., *P. maritima* R. Br., *P. austriaca* Hoess., *P. divaricata* Ait., *P. echinata* Mill., *P. montana* Du Roi, *P. ponderosa* Laws., *P. contorta* Loud., *P. virginiana* Mill., and *P. tæda* L. In the years 1915 and 1916 *Cronartium Comptoniæ* has been received by the writer from various localities on the following new pine hosts: *Pinus densiflora* Sieb. & Zucc. (one locality), *P. jeffreyi* Oreg. Comm. (two localities), *P. laricio* Poir. (one locality), *P. mugho* Poir. (three localities) and *P. resinosa* Aiton (one locality; three other localities are known but no specimens could be secured).

Successful inoculations have been made by the writer and his colleagues named below, for the first time, so far as can be determined from published statements, from several pines to the alternate hosts. Uredinia were produced on plants of *Comptonia asplenifolia* L. with aeciospores from *Pinus tæda* (one test made), *P. austriaca* (2 tests), *P. rigida* (4 tests) *P. mugho* (3 tests), *P. resinosa* (1 test), *P. jeffreyi* (1 test), *P. laricio* (1 test). Uredinia on Comptonia were successfully used to produce uredinia on Comptonia and *Myrica gale*. Uredinia from *Myrica gale* produced uredinia on Comptonia. These are the first successful inoculations with uredinospores to be reported. In the winter of 1914–15 a special effort was made to secure living plants of all the species of Myrica growing in this country. A stock of the following species was obtained largely through the efforts of G. G. Hedgcock and E. P. Meinecke: *Myrica gale* L., *M. californica* Cham., *M. cerifera* L., *M. carolinensis* Mill., *M.*

[1] Spaulding, Perley. Notes on Cronartium Comptoniæ. Phytopath. **3**: 62. F. 1913.
——. Notes on Cronartium Comptoniæ II. Phytopath. **3**: 308-310. D. 1913.
——. Notes on Cronartium Comptoniæ and C. ribicola. Phytopath. **4**: 409. D. 1914.

inodora Bartr., *M. pumila* Michx., as well as *Comptonia asplenifolia*. Mr. G. F. Gravatt and Dr. G. R. Lyman in 1915 made inoculations under the writer's direction. Five different series of inoculations, with aeciospores from as many different species of pine, were made. The results were as follows:

3 plants—*Comptonia asplenifolia*—uredinia produced on all.
7 plants—*Myrica gale*—uredinia produced on all.
9 plants—*Myrica carolinensis*—no infection.
4 plants—*Myrica californica*—no infection.
5 plants—*Myrica inodora*—no infection.
3 plants—*Myrica pumila*—no infection.
4 plants—*Myrica cerifera*—no infection.

A single plant of *M. carolinensis* was inoculated repeatedly in 1916 by the writer without visible results other than yellow spots on the leaves, which are thought to be due to some other cause. Numerous successful inoculations have been made on *Comptonia asplenifolia:* i.e., a total of two in 1912, eleven in 1913, two in 1914, nine in 1915, and fourteen in 1916. A lesser number has been made on *Myrica gale*, which gives much less striking results. The total number of such successful inoculations on *Myrica gale* is: seven in 1915 and four in 1916.

In 1912 the writer began a series of annual observations in an area of several acres near Lake George, New York, quite thickly covered with natural reproduction of *Pinus rigida*, in which *Cronartium Comptoniæ* occurs as a native parasite. The size of the trees within the area ranges from twenty feet in height downward. Very few are as small as two feet in height and there seem to be no very young seedlings now appearing. *Comptonia asplenifolia* grows naturally throughout the area so that conditions are excellent for the spread and development of the disease. These observations have continued until the present time, thus covering a period of five years. An effort has been made to keep numbered labels on all of the diseased trees, but with indifferent success because of curiosity or mischief in people who happened to see them. Several significant facts have been learned however. On pines *Cronartium Comptoniæ* fruits for a period of seven or eight weeks, the time of maximum fruiting being about June first considerably earlier than the writer at first supposed. The number of diseased trees killed annually by the fungus has been surprisingly uniform, ranging from eight to eleven. In 1916 the observations were made earlier than usual and a greater total number of diseased trees were unexpectedly found. There is evidence that a small number of trees are annually infected for the first time and it is hoped to learn this number in the future. It is well established that a tree which once

bears fruit of the fungus almost always bears an annual crop of such fruits until the tree dies. In some cases a dying tree fails to produce them, but on the other hand, recently killed trees are often found with aecia on them. While the annual loss is not great, it is a serious loss when continued indefinitely. Moreover we have excellent reasons for believing that *Pinus rigida*, to which the preceding statements apply, is much less susceptible to the disease than are *P. ponderosa* and *P. contorta*. The loss in some instances, with the latter two species, has been total.[2]

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

[2] Kauffman, C. H. and Mains, E. B. An epidemic of Cronartium Comptoniæ at the Roscommon state nurseries. Mich. Acad. Sci., Ann. Rept. 17: 188-189. 1915

FURTHER NOTE ON A PARASITIC SACCHAROMYCETE OF THE TOMATO

ALBERT SCHNEIDER

Since the appearance of the recent article on a parasitic Saccharomycete of tomato[1] it has been determined that the fungus described unquestionably belongs to the genus Nematospora of Peglion.[2] In its morphological characteristics it is closely similar to *Nematospora Coryli* Pegl., which attacks the fruit of the hazel bush (*Corylus avellana*). Peglion, however, makes no reference to the gametic origin of the ascus nor does he note the two cells of the ascospore. He also fails to recognize the arthrospores and includes them under "Anomale vegetative Formen." These and other morphological as well as biological differences make it clear that the *Nematospora Coryli* of Peglion and the fungus under consideration are two distinct species. Both are true parasites and appear to occur in warmer countries (southern Italy, southern California and Cuba) and perhaps also in semitropical and tropical countries.

The Nematospora of the tomato (*Lycopersicum esculentum*) is apparently a new species and the following name is therefore proposed:

Nematospora Lycopersici n. sp.

Asci of gametic origin soon becoming free from associated cells, cylindrical with rounded ends, 60 to 70μ in length; ascospores in two groups of four spores each, two-celled, slender, with pointed ends, slightly ridged at transverse septum, 50 by 4.5μ; ascospores liberated by dissolution of ascus wall and held together somewhat in groups of 4 by motionless flagellae; flagellae 50 to 100μ in length; arthrospores, of non-gametic origin, spherical to ampulliform, 25μ in diameter. Two other cell forms also found: (1) much elongated, filamentous cells; (2) elliptical and ovoid cells, gametic in function, new cells formed in bipolar direction by apical budding and also by apico-lateral budding at cell unions. The elliptical and ovoid cells alone are gametic in function.

Habitat. Parasitic on nearly ripe and ripe fruit of *Lycopersicum esculentum*, southern California, Cuba and Mexico.

[1] Schneider, Albert. A parasitic Saccharomycete of the tomato. Phytopathology 6: 395-399. 1916.

[2] Peglion, Vittorio. Ueber die Nematospora Coryli Pegl. Centralbl. f. Bakt. Abt. 2, 8: 754-761. 1901.

Nematospora Lycopersici sp. nov.

Ascis cylindratis, terminato orbiculato; 60–70μ in longitudine; sporidiis 8; dispositis struibus duo, sporidiis 4. Ascus mox ex cellis prehensis liberatus est. Tunicae ascorum in maturitate solventur et sporidiis liberantur. Plurimi asci origine gametata sunt.

Sporis ascorum bi-cellulatis tenuibus, fusiformibus, flagello uno. Sporidiis 50 x 4.5μ, flagello 50–100μ.

Arthrosporis non-gametatis, plerumque sphaeroidis, 25μ.

Cellulis vegetativis, ellipticis, ovatis ad filaris nonramosis, multiplicantibus gemmatando apiculo-laterali apiculatoque. Cellulis ellipticis et ovatis solis sunt gametatis.

Hab. In fructo Lycopersici esculenti, terris calidis et tropicis.

Acknowledgments are hereby made to Dr. Roland Thaxter and Prof. H. W. Anderson for calling the writer's attention to the Nematospora of Peglion.

UNIVERSITY OF CALIFORNIA
COLLEGE OF PHARMACY
SAN FRANCISCO, CALIF.

PHYTOPATHOLOGICAL NOTES

Albany conference on white pine blister rust. A joint meeting of the North American Committee for the Control of the Pine Blister Rust and the cooperators of the United States Department of Agriculture was held at Albany, New York, November 20 and 21. The meeting was attended by a representative body of men from the states of New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Virginia, Indiana, Wisconsin and Minnesota, and from the Dominion of Canada and the United States Department of Agriculture. The state foresters, pathologists, and nursery inspectors most interested in the fight for the control of the disease were present.

A brief report from each state and from Canada gave the latest news concerning disease survey and eradication work. The reports show the general epiphytotic of the blister rust in New England and east of the Hudson River, with comparatively few centers of infection in New York, Pennsylvania and New Jersey, one infection center in Ohio, two in Wisconsin, and four in Minnesota. A hasty survey made of the western half of the country failed to show any blister rust of white pine present.

It developed that the matter of control of the blister rust in the New England states resolved itself into the practicability of the eradication of currant and gooseberry bushes on a large scale. The control of the blister rust in the Lake States was shown to be on a somewhat different basis, for in that region the spots of infection known are very few. Here the total eradication of all white pines and Ribes near the infection center is being carried out.

The Committee passed resolutions favoring adequate legislation which would permit states to carry out the eradication or control work necessary, following largely the Sanders model horticultural inspection bill. They also favored adequate appropriations by the states to carry on eradication work and by the Federal Government to carry on the survey and experimental work. The Committee favored a Federal quarantine prohibiting shipment of five-needled pines and all species of Ribes from the eastern half of the country to any part of the country west of Minnesota, Iowa, Missouri, Arkansas and Louisiana. A state quarantine was also recommended prohibiting shipment of five-needled pines and Ribes from infected states to others not infected.

A national law was urged prohibiting the importation of all plants

from any other continent into the United States except through the United States Department of Agriculture solely for scientific and experimental purposes.

ROY G. PIERCE

Corn disease caused by Phyllachora graminis. During the summer of 1915, an apparently undescribed disease of corn was observed by F. L. Stevens in Porto Rico. Leaves were collected from the diseased fields in numerous localities in Porto Rico, and a study of the disease based entirely on this herbarium material has been made at the University of Illinois.

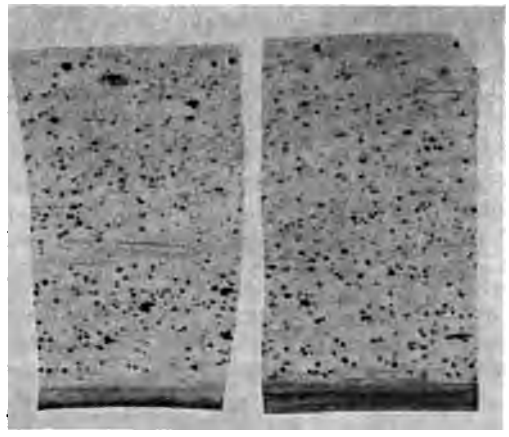

FIG. 1. PHYLLACHORA GRAMINIS ON CORN

Portion of a leaf shows stromata on upper and lower surfaces

The disease manifests itself as well-defined, subcarbonaceous spots, either small and numerous or comparatively large and sparsely distributed, and in either case surrounded by a narrow, yellowish-brown halo. The infection is local, confined to the leaf and the leaf sheaths, and is visible on both upper and lower surfaces.

The spots are due to the formation of stromata in the infected tissue. Embedded in the stromata are perithecia consisting of depressed cavities surrounded by walls made up of dark-brown mycelium and bearing at the top a comparatively small ostiole.

The mycelium in newly invaded tissue is slender and hyaline. It later becomes dark brown, filling the leaf tissue of the infected area with a network of hyphae.

The asci are numerous. They are cylindrical and in each ascus are produced eight unicellular, hyaline, thin-walled spores.

The fungus causing the disease has been identified as *Phyllachora graminis*.

A report including a detailed description of the disease and fungus, together with notes concerning the generic and specific relationship, will be read before the Illinois Academy of Science at its next meeting.

NORA E. DALBEY

Tylenchus tritici on wheat. In August, 1915, specimens of diseased wheat plants, which had just been received from Dr. J. H. Reisner, of the University of Nanking, Nanking, China, were kindly turned over to the writer by Dr. H. B. Humphrey, of the Office of Cereal Investigations, Bureau of Plant Industry. In transmitting this material by letter Dr. Reisner said, "The disease has become more widespread every year for the last three or four years and is causing great money losses."

A microscopic examination of the wheat heads showed that practically all of their glumes contained, in place of normal kernels, dark galls filled with an almost innumerable number of motionless but living larvae of the nematode, *Tylenchus tritici* Bauer, which has been known as a serious pest in Europe since 1745. The parasite has been found in Sweden, Holland, Germany, Austria-Hungary, Switzerland, Italy, England, and Australia, but so far as known has never been reported from China before. Johnson[1] recorded in 1909 the occurrence of what undoubtedly is the same species on wheat from a few widely separated sections in the United States, but as no reports of its appearance before or since that date have been found, it is quite unlikely that the eelworm has become well established in the wheat areas of this country. Whether the closely related species of Tylenchus found by Bessey[2], the writer, and others on several different grasses in various parts of the United States is identical with the form on wheat has not been determined. Some European investigators, however, regard *Tylenchus tritici* as a highly specialized parasite of the wheat.

The infected heads of wheat are usually shorter and thicker than normal heads and contain glumes which spread out almost at right angles to the fruit stem. In place of normal seed, dark, hard galls, incapable of germination and full of larvae, are to be found. Because of these effects on the host the disease has merited such descriptive names as ear-cockles, purples, false ergot, etc.

[1] Johnson, Edw. C. Notes on a nematode in wheat. Science n. s. 30: 576. 1909.
[2] Bessey, Ernest A. A nematode disease of grasses. Science n. s. 21: 391. 1905.

Active larvae enter the young, tender tissues of the wheat flower, extract food therefrom, mature, and lay eggs, which in turn give rise to another generation of larvae. After reaching a certain stage of development some of the subsequent generations of larvae become coiled and dried out in the matured seed coats of the host and are capable of remaining in this inactive condition for long periods. Under favorable conditions of moisture and temperature the eelworms may escape from the seed, attack the leaf and stem parts of wheat seedlings, causing them to become wrinkled, distorted, or swollen, and finally enter the embryonic seeds.

It has seemed desirable to bring the above data to general attention, in the hope that active measures will be taken both to prevent the introduction of this parasite along with wheat importations from infected countries and to stamp out the pest wherever it is found in this country.

L. P. BYARS

The Botanical Society of Washington. The following officers have been elected for the ensuing year:

President, Mr. T. H. Kearney; Vice-President, Mr. Edgar L. Brown; Recording Secretary, Mr. Charles E. Chambliss; Corresponding Secretary, Dr. H. L. Shantz; Treasurer, Mr. F. D. Farrell.

Mr. A. S. Hitchcock was nominated by the Society for the position of Vice-President of the Washington Academy of Sciences.

Personals. Mr. Chas. S. Reddy, of the University of Wisconsin, has been appointed as assistant plant pathologist, and Mr. A. M. Christensen, of the North Dakota Experiment Station, as an agent, in Cereal Disease Investigations, Bureau of Plant Industry, with headquarters at Fargo, North Dakota, where they are engaged in the investigation of cereal diseases in cooperation with the North Dakota Station.

Mr. F. A. McLaughlin, instructor in botany at the Massachusetts Agricultural College, has been granted a year's leave of absence for graduate study at the University of Chicago.

Mr. W. L. Doran, for the last two years graduate assistant in botany at the Massachusetts Agricultural College, has been appointed instructor in botany and assistant botanist at the New Hampshire Agricultural College and Experiment Station.

ABSTRACTS OF PAPERS PRESENTED AT THE EIGHTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, NEW YORK CITY, DECEMBER 26-30, 1916

Evidence of the over wintering of Cronartium ribicola. PERLEY SPAULDING

Numerous instances have been noted where large lots of black currants were very heavily infected with *Cronartium ribicola* one summer and not the next. In the Geneva New York case the disease was present upon pines and these in the writer's opinion started the disease each spring. Cooperative experiments with Stewart, in which 500 heavily infected black currants were used, resulted in no disease. Furthermore, the writer has had during the past seven years in the greenhouses at Washington, hundreds of Ribes plants of more than thirty species, which have been used in inoculation experiments. In no case has the disease ever appeared upon these the next season until artificial inoculations had been made. Uniform success has been obtained personally in locating the diseased pines from the areas occupied by the first generation of uredospores in the field. Field observations seem to indicate that the disease has been shipped into new localities on infected Ribes. Infections of petioles are not as rare as at first supposed. No evidence of bud infection by way of the petiole has yet been secured. Direct examination of buds on infected plants has also failed to show the presence of the disease.

The control of white pine blister rust in small areas. W. H. RANKIN

The control of white pine blister rust has been attempted in eighty-five forest plantings made with imported stock in New York State. Diseased or suspicious trees and all currants and gooseberries within five hundred feet of the plantings were removed. Thirty-six plantings have shown diseased trees since 1909; twenty since 1911; seventeen since 1912; fifteen since 1913; nine since 1914 and four since 1915. The *Ribes* sp. within one-half mile of all the plantings were inspected in the autumn of 1916. Diseased *Ribes* sp. were found around two, only. These were two of the four which had shown diseased trees in the spring. In both cases cultivated varieties of *Ribes* still existed within five hundred feet. It seems, therefore, that the removal of diseased trees and all currants and gooseberries within five hundred feet of the plantings has prevented the establishment of *Cronartium ribicola* in these areas.

Citrus canker investigations at the Florida Tropical Laboratory. R. A. JEHLE

Some cultural characteristics of the canker organism, *Pseudomonas Citri* Hasse (Migula's genus) or *Bacterium Citri* (Cohn's genus Bacterium as emended by Smith) are as follows: Growth on standard agar abundant, spreading, raised, smooth, glistening, transluscent, pale yellow, viscid, with characteristic odor in about five days. Vitality ten to thirty days. Growth on potato agar more abundant and spreading with lighter color. Growth on potato slices more viscid and brighter yellow with distinct white margin on potato adjacent to culture. White margin becomes very prominent in forty-eight hours and does not stain with iodine. On grapefruit leaf and stem decoction agar slants growth penetrates the agar and is

less raised. On oat agar slants growth is much more spreading and the color is much lighter.

Positive results have been obtained from inoculations on grapefruit, ponderosa lemon, key lime, *Citrus trifoliata*, sour orange, tangelo, sweet orange, tangerine, king orange, mandarin-lime, and kumquat. Disease also occurs on navel orange, mandarin, satsuma, common lemon, rough lemon, and *Aegle glutinosa*.

Studies upon the anthracnose of the onion. J. C. WALKER

A morphological study of the causal organism *Colletotrichum circinans* (Berk.) Vogl. shows the fruiting body to be an acervulus and not a pycnidium as first described by Berkeley, confirming the findings of Voglino who transferred the fungus from the genus Vermicularia to Colletotrichum. Further study has shown it to conform closely to the description of *Colletotrichum fructum* (S. & H.) Sacc. (*Volutella fructi* S. & H.), reported by Stevens and Hall as causing a fruit rot of apple. Inoculation of the fungus from onion into apple fruits resulted in a rot very similar to Volutella rot. Further study is necessary before the two fungi can be considered as identical.

Inoculation of onion bulbs in soil held at different temperatures shows best infection to take place between 24° and 29°C. This may account in part for the rather sparing appearance of the disease until shortly before harvest. The fungus over winters in the soil and consequently the disease is most severe on old onion fields.

Spraying the bulbs before harvest or in the crates after harvest has not proved beneficial. The fact that yellow and red varieties of onion are highly resistant offers some encouragement for the development of a resistant white strain. Work in this direction is to be continued.

Pink root, a new root disease of onions in Texas. J. J. TAUBENHAUS and A. D. JOHNSON

A new disease known as pink root is causing serious losses to onion growers of Webb County, Texas. The trouble seems to prevail only where onions are grown for two years or longer on the same land. The same is also true for the seed bed where the same old soil is used for several years in succession. The disease starts with the young sets in the seed bed and from there is carried to the field.

The roots of the affected sets in the seed bed or the plants in the field turn pink in color then strivel and die. As fast as new roots are formed they become infected, turn pink and dry. The effect of pink root is to prevent the normal development of the bulbs in the field and to produce dwarfed undersized bulbs which are absolutely worthless as far as the market is concerned. The cause of the disease is still problematic. Attention is called to it at this time because of its great economic importance to Texas onion growers. Extensive investigations are now under way to determine the cause and possible remedies for this disease.

Two new camphor diseases in Texas. J. J. TAUBENHAUS

Two new or little known diseases seem to threaten the existance of camphor trees in Texas:

1. *Anthracnose.* The fungus attacks and kills the leaves and branches. Affected trees have a defoliated appearance at the top. The cause of the trouble is apparently a new species of Glœosporium tentatively named *Glœosporium camphoræ*. The organism is readily grown in pure culture and the disease reproduced at will.

2. *Limb canker.* This disease is characterized by a dying of the larger limbs to about four to six feet from the top. The limbs turn dark and soon shed their leaves. Affected trees have a ragged and burned appearance. A fungus of the genus Diplodia

is always associated with this disease. Investigations are now under way to determine whether this Diplodia is the same or similar to *D. Camphoræ* F. Tassi, occurring in Italy, and whether also it is the direct cause of the disease. A full description of the two organisms will appear at a later date.

Common and scientific names of plant diseases. M. B. WAITE

Common names of plant diseases are used by a larger number of people than scientific names. Pathologists should encourage the movement to make common names definite and national. By being made definite they can attain their proper status in discussions, literature, dictionaries, quarantine regulations, laws, and legal proceedings. By agreement among pathologists they may even become more fixed than scientific names.

There may be four distinct names connected with every parasitic disease; the common name of the disease, the scientific name of the disease, the common name of the parasite, the scientific name of the parasite. For example: lemon scab, Verrucosis, lemon scab fungus, *Cladosporium Citri*. In case of all common diseases the aim should be to provide these four names. Confusion has resulted in the failure to recognize these four kinds of names, particularly in the use of the scientific name of the disease and the scientific name of the fungus as the common name of the disease. Scientific names may become common names through use but these cases should be clearly recognized as such and avoided if possible and vacancies in names also recognized.

Nonparasitic diseases may have two names, common and scientific, and the same principles apply.

Economic hosts of Sclerotinia libertiana in tidewater Virginia. J. A. MCCLINTOCK

The warm, humid climate of tidewater Virginia is especially favorable to the development of *Sclerotinia libertiana*. This fungus, long known as a serious parasite on lettuce, has been observed to destroy over fifty per cent of the autumn lettuce crop on farms where no rotation is used. In the fall of 1915 a serious disease of snap beans due to this organism was found. During the winter of 1915–1916 *Sclerotinia libertiana* was found to be the cause of a fruit rot of tomato in the greenhouse. In winter-grown parsley, under sashes, this fungus in one case caused drop of ten per cent of the crop in the infected frames. *Sclerotinia libertiana* caused the damping off of a large proportion of the seedling plants in several cold frames of cauliflower being raised for a spring crop of 1916. In the summer of 1916 this fungus caused a stem blight of bearing egg plants, on several farms. In each case the writer was able to isolate the causal organism and to reproduce the disease in the respective hosts, and in other hosts by cross inoculation.

Lima bean mosaic. J. A. MCCLINTOCK

During the summer of 1916 while conducting experiments with nine varieties of pole, and seven varieties of bush lima beans, the writer observed a serious mosaic. It was observed first on the Sieva pole lima or butter-bean and later on Improved Henderson's Bush and Prolific bush, lima beans of the Sieva type.

Over twenty-five per cent of the several hundred plants of each of the above-mentioned varieties were stunted and bore the dwarfed, mottled, wavy leaves, characteristic of this mosaic. None of the varieties of larger limas, which made up the remainder of the planting, showed signs of this mosaic, though they were grown under the same conditions and in many cases intertwined with the mosaic diseased plants

of the Sieva type. Lima beans had not been grown on this land previously and no beans of this type were growing nearby, therefore, it was concluded that this lima bean mosaic was carried by the seed. This disease is serious because the yield on the infected plants is greatly decreased and the pods are smaller and malformed.

Bean mosaic. V. B. STEWART and DONALD REDDICK

Hundreds of acres of pea beans (*Phaseolus vulgaris*) in New York showed the mosaic disease in 1916 and in some fields practically every plant was affected. Affected plants rarely set pods. The disease is not confined to pea beans. Numerous other varieties of dry and snap beans showed the disease but not so commonly as pea beans.

The mosaic-diseased leaves on affected bean plants show irregular crinkled areas, somewhat deeper green in color than the surrounding yellowish green tissue. The disease is transmitted through the seed. Bean seed from mosaic-diseased plants developed diseased seedlings. Healthy seedlings rubbed with crushed mosaic-diseased leaves showed infection four weeks later. The first signs of the disease appeared in leaves which developed about blossoming time. Leaves which had developed previously remained healthy. High temperature and humidity at time of inoculation slightly favor infection.

Two transmissible mosaic diseases of cucumbers. IVAN C. JAGGER

In Phytopathology for April, 1916, there is a group of articles, dealing with a mosaic disease of cucumbers, commonly known as white pickle, which causes a motling of both leaves and fruits. In the vicinity of Rochester, New York, there occurs a second and distinct mosaic disease, which exhibits a mottling of the leaves, but shows no effect on the fruits. The latter disease has been repeatedly transmitted to healthy plants by rubbing with crushed diseased leaves, and has been transmitted to muskmelons and to summer-crookneck squashes. This may be the disease observed by Selby in Ohio and by Stone in Massachusetts.

Bean diseases in New York State in 1916. W. H. BURKHOLDER

An investigation of the diseases of the field bean in western New York begun in 1915 was continued during the summer of 1916. The most serious disease, a dry root rot, caused by a species of Fusarium, was reported last year. Morphologically the pathogene is nearly identical with *Fusarium Martii* Ap. and Wr., although infection was not obtained by inoculation with the latter fungus. The organism winters over in manure where bean straw has been used as feed, and thus may be spread from field to field. There is also evidence that the fungus may live for many years in the soil. The disease was found in practically all of the one hundred and fifty fields visited in western New York. Apparently all varieties of beans are equally susceptible to the disease although certain undesirable types of the white marrow are very resistant. A few individuals of these have been selected for breeding stock.

The blight, caused by *Bacterium Phaseoli*, and the mosaic along with dry weather also aided in reducing the bean crop of 1916. There is some indication that *Bacterium Phaseoli* causes a stem girdling. Anthracnose was destructive in 1915, but caused little damage in 1916.

Do the bacteria of angular leaf spot of cucumber overwinter on the seed? EUBANKS CARSNER

This question was first suggested by the writer's observation in June, 1915, of the occurrence of angular leaf spot in a field on recently cleared land surrounded

by woods near Portsmouth, Virginia. This field was removed at least three or four miles from any other cucumber patch.

In 1916 near Madison, Wisconsin, six separate experimental fields were planted with seed from the same source on land which had not been planted to cucumbers for at least three years. The disease appeared on seedlings in all of these fields and in three of them it was noted on the cotyledons. In three commercial fields in the same vicinity, planted with seed from other sources, the disease did not appear at all in one case and not until late in the season in the other two.

The fact that angular leaf spot appeared on seedlings only in the six fields planted with seed from the one source and not in the other fields in the vicinity furnishes the basis for the working hypothesis that the causal organism is seed-borne, and is opposed to the theory of local overwintering of the organism by means of insects or plant debris.

Infected cucumber fruits in considerable numbers have been seen by the writer in seed fields. The method of securing and cleaning the seed affords ample opportunity for the organisms to reach the seed, and the process includes no operation that would be likely to kill all of the bacteria.

Preliminary notes on a new leaf spot of cucumbers. GEO. A. OSNER

During the seasons of 1915 and 1916, the writer's attention was called to a peculiar leaf spot on cucumbers that was causing more or less damage in a number of fields. The spots varied from two tenths to fifteen millimeters in diameter, the majority of the smaller spots ranging from one to two millimeters and being limited in most cases by the veins of the leaf. The larger spots were white or tinged with brown and with reddish brown areas along the veins of the leaf which gave the spots a characteristic mottled appearance.

The disease was found to be due to a fungus belonging to the Dematiaceæ-Dictyosporæ group of the Hyphomycetes. Its exact generic position has not been determined as yet. The organism was secured in pure culture on string bean agar and successful inoculations have been made on young cucumber plants in the greenhouse, the checks remaining healthy in all cases.

Virulence of different strains of Cladosporium cucumerinum. W. W. GILBERT

A considerable number of strains of the cucumber scab fungus, *Cladosporium cucumerinum*, have been isolated from cucumber fruits from different localities and grown in culture, and many series of inoculation experiments have been made on seedlings and young and old plants. It has been found that these strains vary widely in their ability to infect cucumber plants. Some have failed to produce any infection after many trials, while others are uniformly virulent. One strain in particular has given very striking infection practically every time when favorable conditions were maintained. The virulent strains attack cotyledons and stems of young cucumber seedlings in moist chambers and kill them in two to four days. They also attack and kill very rapidly the younger leaves, stems and growing tips of larger plants under favorable moisture conditions. Under identical conditions the nonvirulent strains failed to produce infection. Similar results were secured when young cucumber fruits were inoculated in moist chambers with different strains of Cladosporium.

Dissemination of the organism of cucumber anthracnose. M. W. GARDNER

In a fairly large acreage of cucumbers and other cucurbits under observation in 1916, the appearance of original centers of anthracnose in only the plots or fields

planted with seed from certain sources pointed suspiciously toward disease introduction with the seed. Subsequent inspection of seed farms revealed the presence of anthracnose on seed fruits and that the processes involved in seed extraction may furnish effective means of seed contamination.

Extensive spread from original centers of infection in the cucumber fields under observation followed periods of heavy rainfall. The principal direction of spread was that of the surface drainage. Plate isolations of the fungus were made from soil near diseased plants. Rows of healthy seedlings exposed to drainage water in diseased fields during heavy rains the first week of September became abundantly infected and many seedlings were killed outright.

Glass tumblers were sunk in the ground at various points in two fields to intercept surface drainage during rains. A successful plate isolation of the fungus was made from water thus collected. Samples collected in one field after rains on September 5 and in the other field after rains on September 12, when sprayed or sprinkled on healthy potted cucumber plants, produced numerous anthracnose lesions.

A bacterial stem and leaf disease of lettuce. NELLIE A. BROWN

A serious stem and leaf disease of lettuce appeared in Beaufort County, South Carolina, 1916. The disease occurred chiefly on two plantations, one of seventeen, the other of nine acres; a conservative estimate of loss on the former was sixty per cent; on the latter ninety per cent. Other plantations within a radius of twenty miles suffered one to ten per cent loss.

The affected plants were wilted, the outer leaves blotched and darkened. Rotting was often rapid. A cross-section of stem at an early stage of disease showed a blue-green color; later stage, brown. Both pith and vascular regions were involved. Later the stem usually became dry and brittle. Moderately diseased plants are darkened in patches in stem, and vascular region. Bacteria filled the cells of the blue-green and brown areas. No fungi were found.

A bacterial organism was isolated which when inoculated into lettuce produced the blue-green color throughout the vascular system and pith, which later became brown. Disease appeared on leaves also. Eight months after isolation this organism, which is yellow on various media and is doubtfully motile, is still infectious. In its morphological and cultural characters it does not correspond with any organism recorded as pathogenic to lettuce.

Black spot of pepper. L. E. MELCHERS and E. E. DALE

In 1915 a striking pathological condition of fruits of peppers was noticed; the disease ranging as high as 45 per cent. A species of Alternaria has been consistently associated with diseased areas. These are slightly sunken, dark colored and not confined to any particular location. Inoculations show that the organism is only a weak pathogene when inoculated into normal tissue. When the pericarp is mechanically (slightly) injured, the fungus becomes established and diseased areas enlarge. There are apparently two ways or combinations of factors in which natural injuries may occur to peppers in the field; (1) injured areas from sun-scald, (2) frost injuries. Artificially injuring the pericarp by means of a burning-glass and applying the fungus superficially, produces a condition which is the counterpart of the symptoms occurring in the field. Varietal resistance is shown by the data of 1916. Sweet peppers are more susceptible than the hot varieties. Among 15 varieties grown, the percentage of disease ran from 0.02 to 2.7 among hot peppers and from 0.4 to 23.07 among the sweet ones, with an average of 13.6 among the latter when sprayed and 11.7 in the unsprayed. Bordeaux sprays do not control the malady. Affected fruit is unsalable.

A sclerotium disease of peppers. WILLIAM H. MARTIN

The disease is characterized by the presence of numerous, minute, black sclerotia throughout the fruit as well as on the seeds. With the exception of a blackening of the epidermis, the disease may pervade the entire interior before any external symptoms are noticed.

The fungus was isolated and grown in pure culture. The pathogenicity of the fungus has been fully established by numerous successful inoculations on both green and ripe fruit, as well as on the plant itself, and by the subsequent re-isolation in pure culture. Reinoculations with this second isolation again produced the typical rot.

The identity of the pathogene has not been satisfactorily determined but there is evidence for the belief that it is *Sclerotium bataticola* Taubenhaus.

Successful cross inoculations have been made on pepper and sweet potato as well as on cucumber, tomato, apple and egg plant.

Dissemination of Bacterium Malvacearum. R. C. FAULWETTER

Through investigations of the means by which *Bacterium Malvacearum* may be disseminated, it has been concluded that wind during rainfall is the most important agent. Neither insects nor seed infection can account for the prevalance of the angular leaf spot caused by it in all varieties of cotton. An inoculation experiment consisting of one row of plants in a field free of the disease was followed within a month by infection of the plants to the east as far as the fourteenth row, and in the next month by the spread of the disease to the west. A second experiment arranged and operating during the second month also showed spread to the west. Practically no disease occurred east or west of uninfected plants in the original rows.

It was demonstrated experimentally that the leaf-surface film during heavy dews contained viable bacteria. It is to be expected these organisms will be present during rains. Westerly winds prevailed during the rains at the time the disease spread to the east, and during the next month the wind direction had changed, blowing from the south-east. Considering the slight disease opposite the uninoculated plants, and the simultaneous change of wind direction and the spread of the disease, it is held that wind during rainfall is the most active agent in the dissemination of the causal organism.

Bacterial diseases of celery. W. S. KROUT

These diseases occur in a region with a deep muck soil and a very humid climate.

Crown rot. This disease is prevalent in the greenhouses and fields. The symptoms are a yellowing of the foliage and a rotting of the crown starting through side roots. Plants are destroyed in from three to four weeks after infection. The causal organisms appear to be a Bacterium and a Fusarium working simultaneously. Steam sterilization and the following chemicals have been applied in varying amounts to the soil for the control of the disease: formalin, calcium chloride, copper sulphate, ferrous sulphate, sulphur, sodium chloride and potash.

Crown rot wilt. This disease is intermittent in its occurrence. It is characterized by a sudden wilting of the entire foliage, an oval hollow cavity in the crown and a badly diseased tap root which serves as a channel of infection.

Bacterial heart wilt. The bacteria attack only the innermost, tender leaves causing a wet, carbonaceous rot. The organism has been isolated and its pathogenicity proved.

A bacterial blight of soy bean. A. G. JOHNSON and FLORENCE M. COERPER

For a number of years this disease has been under investigation at Madison, Wisconsin. A malady apparently the same has also been reported from other parts of the United States. At Madison the disease has been common during the past three years, especially on the leaves.

These leaf lesions are small, rather angular spots, in late stages, dark in color, brown to purplish black. In the earlier stages they are translucent and water soaked in appearance and yellowish to light brown in color. The lesions may be irregularly scattered or variously grouped and they not uncommonly coalesce. Rather inconspicuous glistening films of exudate are frequently noticeable on the lower surfaces of the lesions.

Repeated isolation cultures have yielded a characteristic, white bacterial organism which has proved pathogenic on soy bean, producing characteristic lesions as described above. The same organism has been reisolated from such lesions and its pathogenicity in turn proved. This organism is a rod with rounded ends, motile by a single polar flagellum, hence referable to the genus Pseudomonas of Migula or the genus Bacterium of Ehrenberg as interpreted by Erwin F. Smith.

Studies on the physiological characteristics of the organism and its pathogenicity on other leguminous hosts are in progress.

Host limitations of Septoria Lycopersici. J. B. S. NORTON

Inoculations of seedlings of a number of Solanaceae and eighty varieties of tomato in humid enclosures, with Septoria from tomato resulted in infections on several species of Solanum, eggplant, *Datura tatula*, and especially on potato, currant tomato and *Solanum carolinense*. Spots developed better and spores larger on potato and horse-nettle than on tomato, while the Datura spots were slow-growing, light colored and small-spored. With larger plants outdoors, infections appeared rarely except on Lycopersicum; but undoubted infections resulted on horse-nettle and potato and occasional pycnidia developed with spores smaller than on tomato. The tomato varieties in the seedling stage, showed decided differences in susceptibility to the Septoria, both in number of infections and time of development. Many dwarf varieties showed marked susceptibility.

Wintering of Septoria petroselina var. Apii. W. S. KROUT

Heretofore, the seed has been considered the primary source for dissemination and wintering of this organism. The following observations and results of tests indicate this is not the case: (a) Pycnidia with spores are found on the pedicles and have been reported on seed. (b) All spores taken from the dried pedicles failed to germinate. (c) Spores from green celery tissues subjected to dessication for eight months under laboratory conditions failed to germinate. (d) Young seedlings in the seed-bed were never infected unless planted on soil that had previously grown celery infected with this organism. (e) This organism forms sclerotial-like intercellular bodies in the petioles. (f) Celery grown in new localities gradually becomes infected. (g) Seed from the same bag, but divided and sown upon two different farms produced the diseased seedlings in one case and healthy seedlings in the other.

These studies indicate that the disease is not carried in the seed but in manures containing diseased, decomposed plants, and probably by other methods.

Laboratory work has shown that heating celery seed to 50°C. for one-half hour will eliminate all chances (if there be any) of the disease being disseminated through the seed and pedicles.

Incomplete studies on *Cercospora Apii* Fr., indicate similar conditions.

A nematode disease of the dasheen and its control by hot water treatment. L. P. BYARS

During the summer of 1914, a new disease of an economic aroid, the dasheen, *Colocasia esculenta* (L.) Schott, was found at one point in Florida causing serious damage. The malady is caused by the widely distributed nematode or eel worm, *Heterodera radicicola* (Greef) Müller, which causes root-knot of many wild and cultivated plants, but which has not heretofore been authentically reported on the dasheen. In some places it has caused almost a complete failure of the dasheen crop and is regarded as the most serious pathological factor in the successful production of this plant.

The disease has been found on dasheens in most of the South Atlantic States where economic aroids are grown and in a shipment of cormels imported from Egypt for propagating purposes.

On dasheen roots the nematode produces macroscopically conspicuous swellings which hinder normal absorption. On the surface of the tuberous growth it causes protuberances and definite raised areas resembling open sores, through which secondary field and storage parasites may readily enter. The eelworm does not live on the aerial parts of the dasheen, but, in case of severe infection, it causes these portions to be greatly reduced in size, and gives to the plant as a whole a decidedly sickly appearance.

The disease has been successfully controlled by planting on uninfected land selected cormels from disease-free areas, or diseased cormels which have been treated with water at 50°C. for forty minutes.

Noteworthy Porto Rican plant diseases. F. L. STEVENS

To be printed in full in the April issue of PHYTOPATHOLOGY.

Sulfuring Concord grapes to prevent powdery mildew. F. E. GLADWIN and DONALD REDDICK

Continuing work reported in Internat. Cong. Vit. Off. Rept. **1915**: 117–125, 1916, plats of Concord grape vines were dusted three times, July 18, August 2 and August 16, with sulfur-lime mixtures containing respectively twenty-five, fifty and seventy-five percent sulfur flour, ninety-five per cent or more of which passes 200-mesh sieve. The mixtures were applied at the rate of forty pounds per acre. A single application of Bordeaux mixture was made on one plat on August 11. Treated plats alternated with check plats and all plats were separated by one buffer row.

Powdery mildew, caused by *Uncinula necator*, was abundant. At harvest time one untreated plat showed four per cent of the clusters free from mildew and another only 0.007 per cent free. The bordeaux-sprayed vines showed six per cent of the clusters free from mildew, the mixture containing seventy-five per cent sulfur showed ninety-six percent free, that containing fifty per cent showed eighty-three per cent free and that containing twenty-five per cent showed twenty-seven per cent free. The seventy-five per cent mixture caused severe burning, the fifty per cent mixture a small amount of burning and the twenty-five per cent mixture slight burning

The aecial stage of Coleosporium elephantopodis. GEO. G. HEDGCOCK and W. H. LONG

Young trees of *Pinus heterophylla* in the greenhouse at Washington, D. C., were inoculated under controlled conditions in November, 1915, with the teliospores of *Coleosporium elephantopodis* (Schw.) Thum. In February, 1916, the aecia of *Peridermium carneum* (Bosc.) Seym. and Earle appeared on the needles in abundance.

These were fully mature late in March. Inoculations with the aeciospores March 7 and April 5, 1916, on the leaves of plants of *Elephantopus tomentosus* L. produced in abundance the characteristic uredinia and telia of *Coleosporium elephantopodis*.

During the past three years parallel sets of inoculations of plants of Vernonia on the one hand and of Elephantopus on the other with the aecia of *Peridermium carneum* from a number of species of pine have resulted in producing *Colesoporium vernoniæ* B. and C. on the former, and C. *elephantopodis* on the latter, indicating the identity of the two species.

Peridermium carneum is now reported for the first time on the needles of *Pinus caribæa* Morel., *P. clausa* (Engelm.) Sarg., *P. echinata* Mill., *P. glabra* Walt., *P. heterophylla* (Ell.) Sudw., *P. ponderosa* Laws., *P. rigida* Mill., *P. scopulorum* (Engelm.) Lemm., and *P. serotina* Michx.

The Peridermium belonging to Coleosporium ipomœæ. GEORGE G. HEDGCOCK and N. REX HUNT

Peridermium ipomœæ a new foliicolus species on *Pinus echinata* Mill., *P. palustris* Mill., *P. rigida* Mill., and *P. taeda* L. is described, with a range from Pensylvania to Florida and Texas.

Plants of *Ipomœa lacunosa* L., *I. pandurata* L., *I. triloba* L., *Pharbitis barbigera* (Sims.) G. Don., *P. hederacea* (L.) Choisy, and *Quamoclit coccinea* (L.) Moench under controlled conditions were successfully inoculated with the aeciospores of this Peridermium, producing on their foliage the typical uredinia and telia of *Coleosporium ipomœæ* (Schw.) Burrill, thus proving that it is the aecial stage of this Coleosporium. Plants of species of Amsonia, Aster, Calonyction, Chrysopsis, Convolvulus, Coreopsis, Elephantopus, Helianthus, Laciniaria, Silphium, Solidago, Verbesina and Vernonia were unsuccessfully inoculated.

Coleosporium ipomœæ is now reported for the first time on the leaves of *Ipomolea caroliniana* Pursh., *I. trifida* (H. B. K.) G. Don., and *Pharbitis barbigera*.

A Peridermium belonging to Coleosporium terebinthinaceæ. GEO. G. HEDGCOCK and N. REX HUNT

Peridermium terebinthinaceum, a new foliicolus species on *Pinus echinata* Mill., *P. rigida* Mill., and *P. taeda* L., is described with a range from North Carolina to Georgia.

Inoculations were made under controlled conditions with the aeciospores of this Peridermium on plants of *Silphium asteriscus* L., *S. integrifolium* Michx., *S. trifoliatum* L. and *Parthenium integrifolium* L. in May and June 1916. In about two weeks the uredinia, and later the telia of *Colesporium terebinthinaceæ* (Schw.) Arthur appeared on the leaves of all these species, proving the Peridermium to be the aecial stage of this Coleosporium. Inoculations were made at the same time on plants of species of Amsonia, Coreopsis and Laciniaria without result.

Coleosporium terebinthinaceæ is now reported for the first time on the leaves of *Silphium angustum* (A. Gray) Small, *S. compositum* Michx., *S. dentatum* Ell., *S. glabrum* Eggert, and *S. pinnatifidum* Ell.

An alternate form for Coleosporium helianthi. GEORGE G. HEDGCOCK and N. REX HUNT

A new foliicolus species, *Peridermium helianthi*, is described on *Pinus virginiana* Mill., with a range from Pennsylvania to South Carolina and Tennessee. Inoculations made with the aeciospores of the Peridermium, under controlled conditions,

on plants of *Helianthus decapetalus* L., *H. divaricatus* L., *H. giganteus* L., *H. glaucus* Small, and *H. hirsutus* Raf. produced the uredinia and telia of *Coleosporium helianthi* (Schw.) Arthur, usually in abundance, thus proving the Peridermium to be the aecial stage of this Coleosporium. Inoculations were made at the same time on plants of species of Aster, Chrysopsis, Coreopsis, Elephantopus, Laciniaria, Parthenium, Rudbeckia, Silphium, Solidago, Verbesina, and Vernonia with negative results. The results of these inoculations indicate that the Coleosporiums on Coreopsis and Verbesina are distinct from the one on Helianthus. The Coleosporium in Florida on Verbesina which has been assigned to *C. helianthi* may belong to one of the unnamed species of Peridermium found by the writers in the south.

Coleosporium helianthi is now reported for the first time on *Helianthus australis* Small, *H. divaricatus* L., *H. eggertii* Small, *H. glaucus* Small, *H. grosse-serratus* Martens, *H. hirsutus* Raf., *H. microcephalus* T. and G. ,and *H. saxicola* Small, and its range (on Helianthus) is extended to Louisiana and Florida.

Some new hosts for Coleosporium solidaginis. GEORGE G. HEDGCOCK and N. REX HUNT

Peridermium acicolum Underw. and Earle, the aecial stage of *Coleosporium solidaginis* (Schw.) Thüm., is reported for the first time on *Pinus caribaea* Morel., *P. contorta* Loud., *P. divaricata* (Ait.) Du Mont de Cours, *P. echinata* Mill., *P. elliottii* Engelm., *P. mayriana* Sudw., *P. nigra* Arnold (*P. laricio* Poir.), *P. nigra* var. *austriaca* Schneid., *P. palustris* Mill., *P. ponderosa* Laws., *P. scopulorum* (Engelm.) Lemm., *P. serotina* Michx., *P. taeda* L., and *P. thunbergii* Parl.

Positive results have been obtained from inoculations with the aeciospores of *Peridermium acicolum* on plants of species of Aster and Solidago, and negative results only, on plants of species of Campanula, Convolvulus, Coreopsis, Elephantopus, Eupatorium, Euthamia, Helianthus, Ipomoea, Laciniaria, Parthenium, Pharbitis, Senecio, Verbesina, and Vernonia. A Coleosporium on *Chrysopsis mariana* (L.) Nutt., found first by W. H. Long in Florida, is tentatively assigned to this species.

The range of the Peridermium has been extended to include nearly all States from Minnesota and New Hampshire on the north to Arkansas and Florida on the south.

Notes on some species of Coleosporium. GEORGE G. HEDGCOCK and N. REX HUNT

Coleosporium delicatulum Arth. and Kern is reported for the first time on *Euthamia caroliniana* (L.) Greene, and *E. leptocephala* (T. and G.) Greene, and *Peridermium delicatulum* (Arth. and Kern) Hedge. and Long for the first time on *Pinus caribea* Morel., *P. echinata* Mill., *P. elliottii* Engelm., *P. heterophylla* (Ell.) Sudw., *P. mayriana* Sudw., *P. palustris* Mill., *P. nigra* Arnold, *P. ponderosa* Laws., *P. resinosa* Ait., *P. serotina* Michx., and *P. taeda* L., and the range of the species is greatly extended.

Coleosporium laciniariæ Arth. is reported for the first time on *Laciniaria earlei* Greene, *L. elegans* (Walt.) Kuntze, *L. elongata* Greene, *L. pauciflora* (Pursh) Kuntze, *L. scariosa* (L.) Hill, and *L. serotina* Greene, and the range of the species extended to Florida on the South and to New Jersey on the north.

Some new hosts for Coleosporium inconspicuum. GEORGE G. HEDGCOCK and N. REX HUNT

Aeciospores from *Peridermium inconspicuum* Long collected for the first time on the needles of *Pinus echinata* Mill. were successfully inoculated on the leaves of

both *Coreopsis major æmleri* (Ell.) Britton and *C. verticillata* L. resulting in the formation of the uredinia and tellia of *Coleosporium inconspicuum* (Long) Hedgc. and Long. Unsuccessful inoculations were made on plants of species of Amsonia, Aster, Chrysopsis, Elephantopus, Euthamia, Helianthus, Laciniaria, Silphium, Solidago, Verbesina, and Vernonia. The results of these inoculations indicate that *Coleosporium inconspicuum* is distinct from species found on plants of these genera. This Coleosporium is reported for the first time on *Coreopsis delphinifolia* Lam., *C. lanceolata* L., *C. major* Walt., *C. major rigida* (Nutt.) Boynton, and *C. tripteris* L.

Coleosporium apocynaceum Cooke has been collected at Clearwater, South Carolina on *Amsonia cliate* Walt.

Scolecotrichum graminis on timothy, orchard grass, and other grasses. A. G. JOHNSON and C. W. HUNGERFORD

During the past few years *Scolecotrichum graminis* has been observed by the writers on timothy and orchard-grass at various points from Wisconsin to the Pacific Coast. The fungus causes a serious disease of these hosts especially in Wisconsin. The young lesions on the leaf are circular to elliptical in form, vary greatly in size, and are usually purplish brown in color, The older lesions turn grayish brown as the invaded tissues die. In severe cases they coalesce involving considerable portions of the leaf-blades. In the worst cases practically all of the leaves of affected plants are dried up at about flowering time. In moist weather the fungus sporulates abundantly on the older lesions. On orchard grass, the lesions are somewhat more distinctive and sporulation takes place more abundantly. The conidiophores come out through the stomata and form dark-colored tufts arranged rather regularly in rows.

Other grasses observed by the writers as hosts for the fungus are *Agrostis alba, Bromus marginatus, Bromus sitchensis, Hordeum jubatum, Hordeum nodosum, Elymus glaucus* and *Elymus robustus.*

Observations at Madison have shown that the fungus over-winters readily in tufts of orchard grass and timothy and resumes activity early in the spring.

Bacteria of barley blight seed-borne. L. R. JONES, A. G. JOHNSON, and C. S. REDDY

In further studies on the bacterial blight of barley, upon which reports have been previously made, the mode of overwintering of the causal organism and of its dissemination over long distances have received especial attention. Field evidence early indicated that in certain cases the disease doubtless was introduced with seed from various western sources. In following up this matter, seed was collected in 1914 from a field of barley in Montana severely affected by the blight. Lesions were evident on the glumes of these plants before maturity and showed, although less clearly, upon the ripe grain. Some of this seed was planted in our trial grounds in Wisconsin in 1915, and the blight developed abundantly upon the leaves of the young plants.

Isolation cultures were made in July, 1916, from the glumes of barley kernels from this same 1914 Montana collection. The characteristic barley blight organism was obtained and its pathogenicity proved by inoculation experiments. It is thus apparent that the organism may be carried with the seed grain and remains viable after at least two years of dormancy. Preliminary trials indicate that the organism may be destroyed by seed disinfection.

The Pseudopeziza leaf spot diseases of alfalfa and red clover. FRED REUEL JONES

The Pseudopeziza leaf spots of alfalfa and red clover have been studied for the past two years for the purpose of determining the following points.

1. Are the causal organisms the same or distinct species?
2. Is any other spore-form than the ascospore included in the life history of these fungi?
3. What is the relation of these fungi to the tissues of their hosts?
4. How do these fungi overwinter?
5. Can the occurrence of the alfalfa leaf spot on alfalfa sown in a new region for the first time be prevented by seed treatment?

Progress has been made as follows:

1. Both fungi have been obtained in pure culture. Slight morphological and distinct physiological differences have been found.
2. Only ascospores have been found produced in nature. Conidia-like structures occur in cultures.
3. Germinating ascospores penetrate the epidermal cells directly and the mycelium develops within the host cells and penetrates the cell walls.
4. The fungus overwinters on dead leaves which escape decay, and ascospores developed either in old or new apothecia are a source of spring infection.
5. Alfalfa seed very thoroughly disinfected has been sown at distances up to 15 miles from other alfalfa. Leaf spot has occurred on all these plots.

The development of the aecial stage of Nigredo on red clover. I. E. MELHUS and WILLIAM DIEHL

The occurrence of the uredino- and teliospore stages of *Nigredo fallens* (Desm.) Arthur (*Uromyces fallens* (Desm.) Kern) on red clover (*Trifolium pratense*) is common, but the aecial stage of this rust is apparently not well known. The uredinospore stage developed abundantly on red clover growing in the greenhouse beginning about January 10, 1916, when the clover plants were about six inches tall. These grew in pots forming a border nearest the glass on a bench in a house where the temperature ranged from 10° at night to 20°C. in the day time. On March 26, 1916, aecia were observed on the leaves of the red clover plants. During the next two weeks the aecial stage became abundant. It continued to develop for about a month when the temperature raised in the house due to the increased sunshine. White clover (*Trifolium repens*), alsike (*T. hybridum*), and crimson clover (*T. incarnatum*), growing in close proximity were continually free from infection.

Repeated attempts were made to transfer the rust to the above hosts by using the aeciospores but no infections were obtained except on the red clover. It would appear that *Nigredo fallens* is autoecious and not heteroecious as heretofore reported.

A malnutrition disease of the Irish potato and its control. H. A. EDSON and OSWALD SCHREINER

Beginning early in the past July, potato plants in numerous eastern fields from Maine to Virginia developed a downward curling of the leaf margins accompanied by a bronzing and later a browning but not a yellowing of the foliage. Death of the leaves and sudden collapse of the stems at the ground level followed. Fungi of parasitic habits appeared at and above the point of collapse. Dr. W. A. Orton observed the disease in New Jersey and noted its absence from areas treated with potash or stable manure. Similar observations were made elsewhere, particularly in Maine, where Dr. Joseph Rosenbaum and B. E. Brown and L. A. Hurst have

undertaken a study of the disease in cooperation with the Maine Agricultural Experiment Station. These investigations are incomplete but present indications are that the fungi isolated are weakling parasites operating as contributing factors and that the primary cause is malnutrition, resulting from insufficient potash or perhaps an excess of nitrates in the presence of a minimum potash supply. In Maine the disease appears to be correlated with certain soil types and is most marked on Irish cobbler, Bliss and Eureka, though not entirely restricted to these early varieties. Stable manure is an excellent corrective.

Notes on curly dwarf symptoms on Irish potatoes. W. L. DURRELL

Plants showing curly dwarf symptoms were very prevalent in Iowa this past season on the varieties Irish cobbler, Rural new yorker and Early ohio. In some cases these symptoms were on plants grown from the progeny of plants having shown curly dwarf symptoms the preceding year; in others they were induced by climatic conditions. The disease made its appearance on the early planting about June 10 and continued to develop throughout the season. In August, plants that had been normal up until that time, showed typical signs of curly dwarf on the foliage produced during this period. The upper third of the plant had shortened internodes, crinkled and curled leaves, giving this portion of the plant a bushy appearance so characteristic of curly dwarf. These symptoms were induced in the field by the hot dry weather during August and similar ones were later artificially developed in the laboratory. The plants manifesting these induced symptoms of curly dwarf put forth normal foliage again in September with the advent of cooler weather.

Histological studies show that the crinkling of the leaves is due to necrosis of certain epidermal and cortical cells of the veins, followed by the growth of the parenchyma cells which induces a buckling of the leaf surface. In addition the leaves showing curly dwarf symptoms were characterized by a most marked decrease from the normal in the size of the parenchyma cells. Furthermore transpiration tests using the cobalt chloride paper and potometer methods indicate that curly dwarf plants transpire more rapidly than normal ones.

Notes on mosaic symptoms of irish potatoes. I. E. MELHUS

The so-called mosaic disease of potatoes is characterized by yellow mottling and crinkling of the foliage. Its effect on the potato plant, transmissibility, and relation to curly dwarf is very imperfectly understood. The varieties of Bliss triumph and Green mountain, which showed mosaic symptoms in 1914 and 1915 in northern Maine, were planted in 1916 at Ames, Iowa. The characteristic yellow mottling typical of this disease did not develop at any time during the growing season. Curly dwarf symptoms, however, were prevailingly present.

(a) Plants seemingly badly affected in 1915 produced 0.237 pounds per hill.

(b) Plants seemingly moderately affected in 1915 produced 0.29 pounds per hill.

(c) Plants seemingly slightly affected in 1915 produced 0.32 pounds per hill.

Healthy plants used as checks produced 0.46 pounds per hill.

None of the plants in lots *a* or *b* grew as large as those in lot *c*, but some of the *c*-plants were like those of *a*. Although the typical mottling characteristic of mosaic in certain northern potato growing districts may not develop, the progeny of plants showing these symptoms are undesirable for seed purposes.

Frost necrosis of potato tubers. L. R. JONES and ERNEST BAILEY

A peculiar type of non-inheritable "net necrosis" of potato tubers has been under observation for several years under conditions suggesting frost injury. Carefully

repeated chilling experiments confirm this hypothesis. Tubers "frozen solid" are totally killed and collapse when thawed. If, however, the chilling stops with incipient ice-crystallization, killing may be confined to such interior tissues as are most sensitive. Such chilled tubers appear normal externally but when cut show the interior vascular regions to be most sensitive and hence the first to succumb and discolor. Therefore, moderate exposure to freezing temperature may produce either "ring" or "net" necrosis, the blackened vascular portions permeating the starchy fundamental tissues. Individual variations in sensitiveness occur between tubers, but in general the best types of "net necrosis" have been secured by about two hours exposure to +5°C., with similar results by exposures ranging from −1°C. for 8.5 hours to −9°C. for one hour. Slightly more severe treatments, or unequal exposures may give frozen spots with corresponding dark blotches involving the general parenchyma. The stem end of the tuber is always more sensitive than the other.

Will Spongospora subterranea prove serious in Virginia? J. A. MCCLINTOCK

Potatoes affected with powdery scab planted in Virginia in the spring of 1915 produced a crop free from this disease. In the spring of 1916 affected tubers from Maine were planted at Norfolk, Virginia, and at Tasley, on the Eastern Shore of Virginia. The writer examined the crop at each place but found no signs of Spongospora infection on any of the harvested tubers. Some of the infected seed from Maine was held over summer in cold storage and planted at Norfolk about the time the fall crop of Irish potatoes was planted in Virginia. On November 23, 1916, the tubers were harvested and examined but no Spongospora infection was observed. These results corroborate those of 1915 and lead one to conclude that powdery scab will not be prevalent either on the spring or fall planted potatoes even though the seed tubers are infected with *Spongospora subterranea*.

Seed potato certification in Nova Scotia. PAUL A. MURPHY

Many fields of Garnet chili potatoes for the Bermuda seed trade were infected to the extent of fifty per cent with leaf roll, and whole districts to not less than ten per cent, making a difficult situation when we took charge. Hill selection in districts proved useless in several cases tried. The scheme adopted had to be a comprehensive and unusual one, an outline of which follows.

It is necessary for growers to start with stock of good previous record. This provision, which is absolutely insisted on, is becoming more stringent, and in two years the growers will use exclusively stock which is now selected and is being given a three years' trial. Thereafter it will still be continuously selected, one man being appointed in each district to grow it for his neigbors.

The summer inspection is made jointly by officers of the Departments of Agriculture of Canada and Bermuda, while the autumn inspection is made by the Canadian authorities. The grower's name appears on each barrel, whereby many stocks are traced to the Bermuda plntations. As a further safeguard a sample of every grower's potatoes are sent to Bermuda, where they are all planted together.

The economic importance of mosaic of potato. PAUL A. MURPHY

The beginning of a series of experiments to investigate the economic importance of this disease has given striking results. In one experiment of eleven similar plots side by side, planted partly with healthy, and partly with mosaic-diseased Green mountains of the same strain, the diseased plants gave a yield which was on the

average only 57.8 per cent that of the normal plants, the limits being 52.0 per cent and 63.6 per cent. Furthermore the crop of the mosaic-diseased plants was marketable only to the extent of 82.7 per cent (limits, 74.1 and 87.3), while 91.6 per cent of the crop of the healthy plants was marketable (limits, 81.9 and 94.1). This means that in an average crop of 300 bushels there is a loss of one and one-third bushels of marketable potatoes for every 1 per cent of mosaic present. This coupled with the fact that the trouble is constant every year and that it generally affects, where present, not far from twenty per cent of the crop means a steady loss of from twenty to thirty bushels of potatoes per acre per year. The eating qualities of the potatoes are not impaired.

A new strain of Puccinia graminis. E. C. STAKMAN and F. J. PIEMEISEL

A rust which behaves differently from any of the common biologic forms of *Puccinia graminis* has recently been found on club wheat and a number of wild grasses. It resembles *P. graminis tritici* morphologically and parasitically more that it does any other biologic form. However, the common *Triticum vulgare* wheats which have been inoculated are highly resistant to it. Both *P. graminis tritici* and the new strain have a number of hosts in common, viz: *Triticum compactum*, Barley, *Agropyron smithii*, *Elymus canadensis*, *Elymus macounii*, and *Hordeum jubatum*. The new strain has also been found in nature on *Elymus glaucus* and *E. condensatus* and has infected a number of grasses in the greenhouse. Extensive cross-inoculation experiments are now under way.

The rust was found only west of the Rocky Mountains in Idaho and Washington where it seemed to take the place of ordinary *P. graminis tritici*, none of which was found in the region mentioned.

Puccinia graminis on wheat kernels and its relation to subsequent infection. CHAS. W. HUNGERFORD

Various workers have noted the occurrence of rust pustules on seeds of different grains and some have held that the fungus might infect the plant by this means. Experiments have been carried on at Madison, Wisconsin the last year to determine if possible whether *Puccinia graminis* is able to infect wheat through the seed. Three lines of attack have been followed. (a) Rusted seed after being germinated at different temperatures has been fixed and examined by histological methods and in no case was the fungus found to penetrate the embryonic tissues. (b) Treated and untreated samples of rusted seed, as well as clean seed, were planted in the field and the first appearance of stem rust upon the plants in the different plots was noted at practically the same time. (c) Two lots of rust-infected seed have been grown to maturity in an isolated room in the greenhouse. No rust has appeared on any of these plants. Although the work has not been fully completed, the results so far tend to show that seed wheat infected with *Puccinia graminis* does not cause infection of the wheat plant.

Similar experiments are being started at Corvallis, Oregon, with wheat infected with *Puccinia glumarum*.

Ecological observations on Ustilago Zeae. ALDEN A. POTTER and LEO E. MELCHERS

Pammel and Stewart in 1893 observed that the nodal buds of maize were particularly subject to smut and that "where one smut boil made its appearance on the lower nodes, others appeared further up." It thus becomes desirable to explain how the infection, shown by Brefeld to be strictly local in its development, can spread on the plant. The basis of study has been Brefeld's idea of distribution by

air-borne conidia. The organism has been isolated in pure, conidial culture, both from the air and from the young plants some little time before the disease appeared. The corn plant is thus seen to be well adapted as a spore trap. The conidia caught probably do not infect directly. The result is rather the development of a virulent culture in the leaf axil. A plant may thus become a center of aerial distribution; or, when rain recurs, the conidia may be washed down or splashed out upon other leaves. Thus it may sometimes happen that all the culms of a plant, or hill, will show many nodal infections when an equal number of stalks immediately adjacent will not be infected at all.

The short-cycled Uromyces of North America. G. R. BISBY

Only eleven species of short-cycled Uromyces have been found in North America. These rusts are parasitic upon six families of Monocotyledons and Dicotyledons. Various relationships are evident between these rusts and other long-cycled and short-cycled species of rusts. Seven species are commonly micro-forms; for four of these, pycnia are known. Seven species have strictly local mycelium. These rusts occur mainly in Western and Southern North America. The specimens have been studied at the Arthur Herbarium.

Boleti and mycorhiza upon forest trees and an unusual mycorhiza upon white oak. L. H. PENNINGTON

One instance of a Boletus, *B. speciosus* Frost, connected with mycorhiza of oak was reported in 1908. Since that time five other species, *B. frostii* Russell, *B. indecisus* Pk. *B. chromapes* Frost, *B. purpureus* Fr. and *B. gracilis* Pk. have been found connected with mycorhiza of forest trees, usually oaks. Two of these species, *B. frostii* and *B. indecisus*, have been found to produce sclerotia similar to those reported for *B. speciosus*.

A peculiar form of mycorhiza was found upon white-oak roots in which the hypertrophied branches are closely aggregated and surrounded by a peridium-like layer of fungal tissue. This gives them the appearance of white root tubercles, four to twelve millimeters in diameter. These tubercle-like growths are not unlike those upon beech roots described in 1899 by Von Schrenk. They are also definitely connected with small sclerotia from which there is a growth of mycelium in early summer to produce new mycorhiza upon the roots. Attempts to inoculate the roots of other trees with this fungus have thus far failed.

A new parasitic slime mold suitable for class work. JOHN A. ELLIOTT

The sweet potato "pox" organism, *Cystospora batata* Elliott, as it occurs on sweet potatoes, offers itself as an excellent example of the Plasmodiophorales for use in the laboratory. Infected plants growing between sheets of moist blotting paper afford an abundance of parasitized rootlets and growing points of stems for free-hand sectioning or for embedding in paraffin. Such material is easily sectioned and contains great numbers of the parasite in all stages of its life cycle. The rapidity with which the organism goes through its complete life history makes a study of living material of special value.

Strains of Rhizoctonia. J. ROSENBAUM and M. SHAPAVALOV

During the summer of 1916 a strain of Rhizoctonia was isolated from potato stems which showed a girdling and hollowing at or near the surface of the ground. This

strain, designated R 5, differs in the following particulars from other strains isolated from stems and tubers of potatoes grown in Maine and Florida:

(1) Inoculations with R 5 produced definite lesions in injured potato stems growing in the field and greenhouse and injured tubers, while in the case of inoculations with other strains the lesions, if produced at all, were smaller and the results not so conclusive. The injured checks remained healthy.

(2) Macroscopically R 5 can be distinguished by the darker coloration of the medium, especially when grown on potato agar, and by the light grayish sclerotia as compared with the dark-brown sclerotia of the others when grown on corn-meal agar.

(3) Microscopically R 5 differs from the other strains in its finer mycelium, which measures 5 to 9 μ in diameter while the others measure 10 to 14 μ.

Is it not possible that different strains of Rhizoctonia may offer an explanation for the conflicting reports regarding artificial infection?

The aecial stage of the red clover rust. W. H. DAVIS and A. G. JOHNSON

The well known red clover rust, *Uromyces fallens* (Desm.) Kern, has long been suspected of having an aecial stage. Our observations and experiments have thrown definite light on the question.

Aecia on red clover were first obtained experimentally in the greenhouse in December 1915 and similarly again in January and February, 1916. Later in the spring a number of cases of aecia were observed on the same host out of doors near Madison. Following various sowings of aeciospores from such sources on rust-free red clover plants under glass, uredinospores developed uniformly. These were identical with those commonly observed on that host. Sowings of viable teliospores resulted in the development of aecia, identical with those observed in greenhouse and field.

It is thus evident that this rust is a long-cycled autoecious species, i.e., with pycnia, aecia, uredinia and telia on the same host.

Observations on pear blight in Illinois. F. L. STEVENS, W. A. RUTH, G. L. PELTIER, and J. R. MALLOCH

Experiments made by applying *Bacillus amylovorous* in suspension in water to pear buds in 1915 did not indicate in 1916 that the bacilli hibernated in the buds.

Subcuticular infections of spurs from hold-over trunk cankers occurred in 1916, with a maximum number of twelve such infections from one canker. The organism appeared to be dead in all twig cankers. A few living cankers provided exudate for serious well-distributed blossom infection, which in turn provided exudate for further infections, these continuing until early in June.

Leaves appeared to be at no time naturally infected from the exterior and on June 1 blades and pedicles could not be inoculated though the fruit and pedicles were still susceptible. Bordeaux mixture controlled the floral infection without reducing the set of fruit.

Second progress report on investigations of leaf spot of cherries and plums in Wisconsin. G. W. KEITT

Comparative studies of *Coccomyces hiemalis* Higgins and related organisms in connection with leaf spot diseases of cherries and plums have been continued, and spraying and sanitation experiments in the control of cherry leaf spot have been begun. Only the control work is reported here.

Spraying (Montmorency and Early Richmond). In early summer, the disease occurred in unusual severity, but, after the advent of hot dry weather in late July,

it made relatively little progress. It was satisfactorily controlled by Bordeaux mixture, 4–4–50, 3–3–50, and 2–2–50, and lime-sulphur (commercial concentrate, 33°B.), 1–40, applied (1) when the petals fell, (2) 16–17 days later, and (3) just after the fruit was harvested. "Atomic sulphur," 5–50; barium-sulphur, 3–50; and self-boiled lime-sulphur, 8–8–50, in parallel applications, did not control the disease satisfactorily. An additional application just before the blossoms opened did not increase the efficiency of leaf spot control.

Sanitation. Life history studies, sanitation experiments, and extensive observations indicate that, under Wisconsin conditions, the spray schedule may be strongly reinforced by turning under the fallen leaves as completely as feasible by clean cultivation before the blossoms open (In 1916, the first ascospore discharge was observed as cherry blossoms began to open).

Jonathan spot. CHARLES BROOKS and J. S. COLLEY

The development of Jonathan spot increases with an increase in temperature up to 20°C., but is entirely inhibited at 30°C. The disease can be readily produced in saturated air in closed moist chambers but seldom develops in a stirred air of 70 per cent or 95 per cent relative humidity.

Temperature relations of apple rot fungi. CHARLES BROOKS and J. S. COLLEY

Most apple-rot fungi will grow at a lower temperature on corn meal agar than on fruit and at a lower temperature on ripe fruit than on green fruit. With several if not all of the storage-rot fungi the initial stages of rotting are more inhibited by low temperatures than is the germination of the spores. Rots may finally make a fairly rapid development at temperatures at which the fungus is at first barely able to make a start. Even at favorable temperatures most of the fungi pass through a period of incubation on apples that is not evident on culture media.

Control of apple scab by bleaching powder. W. S. BROCK and W. A. RUTH

Bleaching powder when applied to apple trees in 1916 reduced apple scab from 50 per cent to 11.2 per cent. No injury resulted to fruit or foliage. The leaves on trees sprayed with this material were practically free from scab. Leaves on unsprayed trees showed serious scab infection.

In 1910 the material was applied at high concentrations alone, and with lime, causing little foliage injury, but no scab developed. Higher concentrations alone and in combination with other materials will be tried.

Studies on peach yellows and little peach. M. A. BLAKE, MEL. T. COOK and C. A. SCHWARZE

Symptoms very similar to peach yellows and little peach may be due to other causes. Tests with healthy and diseased trees showed (1) pulp from healthy leaves retains original color longer than pulp from diseased leaves; (2) juice from healthy leaves is more mucilaginous than that from diseased leaves; (3) juice from fast-growing trees more mucilaginous than juice from the leaves of slow-growing trees; (4) leaves taken from healthy trees after sunset showed a minimum amount of starch, leaves from little peach trees a larger amount, and leaves from yellows trees and from girdled trees the greatest amount; (5) leaves from an apparently healthy branch adjacent to a diseased branch on same tree showed a higher starch content than leaves from a healthy tree; (7) leaves from fast-growing trees lose starch more rapidly than leaves from slow-growing trees; (8) juice extracted from healthy

leaves showed less oxidase than juice from diseased leaves; (9) juice from healthy kernels showed less catalase and acid than juice from diseased kernels; (10) the tannin content of healthy fruit is less than that of diseased or forced fruit.

Pits from diseased trees failed to germinate. Budding experiments with diseased buds indicate that the appearance of the disease in the young trees varies with source of bud wood.

A Xylaria root-rot of the apple. F. D. FROMME and H. E. THOMAS

A destructive root-rot of apple trees is prevalent in the chief orchard sections of Virginia. The infectiousness of this condition is shown in the death of adjoining trees in groups irrespective of soil conditions or topography, and in the death of replants set in holes from which diseased trees were removed. Isolations from affected roots from a number of orchards have yielded cultures of a fungus which is apparently the conidial stage of a species of Xylaria. Perithecial stromata of *Xylaria polymorpha* have been found on roots of apple trees showing typical attack. Typical root-rot lesions have been produced with pure cultures of the Xylaria introduced into bark wounds of living apple roots in damp chambers and in the field. The introduced fungus has been recovered in pure culture from these lesions. Two or more species of Xylaria may be involved; their interrelations are subject for further study. Apparently all varieties of apples are susceptible and probably equally so. Observations indicate that the organism may be spread in cultivation, in the removal of borers, in contact between root systems of adjoining trees and in surface washing of infective material.

Pycnial scars, an important diagnostic character for the white pine blister rust. REGINALD H. COLLEY

After the pycnospores appear in their characteristic sweetish drops the whole pycnium is cut out by a protective layer of tissue which forms at a depth of several cells below the pycnial layer. Everything above the protective layer drys out and dies. The result of this drying is a rusty brown patch or scar which indicates by its size the extent of the pycnium. The scars are dark brown and glossy at first. Later they become rusty brown with a dry dusty-granular surface. They average about four millimeters in diameter. Pycnial scars are a positive diagnostic field character for the white pine blister rust, here reported for the first time. They are especially valuable when the bark is but little swollen, and when there is no indication of aecia or of aecial scars.

Mycelium of the white pine blister rust. REGINALD H. COLLEY

The uninucleate mycelium of *Cronartium ribicola* forces its way between the bark cells of white pine, frequently forming strands. As the cells are forced apart the bark swells. Haustoria penetrate practically every non-woody cell in the infected tissue. The sieve tubes become plugged. Hyphae follow the ray cells past the cambium and into the wood for a distance of at least three annual rings. Thus the fungus derives nourishment from both the ascending and descending currents of sap. The morphological characters of the mycelium are definite and constant under all conditions observed. Therefore the mycelium has positive diagnostic value for the blister rust before there is any exterior indication of spore formation.

The binucleate mycelium in Ribes leaves is limited in extent. Haustoria are rare. Uredinia and telia form quickly and burst through the epidermis easily.

The binucleate mycelium is very abundant in cases of petiole infection and the haustoria are larger and more numerous than in the lamina. Normal telia are usually produced but sometimes they are formed internally.

A species of Chrysomyxa new to North America. H. S. JACKSON

The genus Chrysomyxa was established in 1840 by Unger with *C. abietis* (Wallr.) Ung. as the type species, and has generally been interpreted as including both long and short cycle forms. Arthur restricted this genus to include only the microforms and established Melampsoropsis (Schröt.) Arth. for those species with a long life cycle. All of the latter forms are assumed to be heteroecious and have their uredinia and telia on Pyrolaceae, Ericaceae and Vacciniaceae. The aecial stages so far as determined, have proved to be species of Peridermium on Picea. In America eight species have been reported, all of which are long cycle forms and are referred to Melampsoropsis by Arthur. Four of these have been definitely connected through cultures by European and American students with their aecial stages.

A short cycle form referable to the genus Chrysomyxa (as restricted by Arthur) is recognized in America for the first time and causes a disease of the leaves of *Picea engelmannii*.

A Gnomonia on eggplant. C. W. EDGERTON

During the past three years, a species of Gnomonia has been found on old eggplant stems during the winter season at Baton Rouge, Louisiana. This fungus has been repeatedly cultured and it has been found to be very similar, if not identical, from a morphological standpoint, with the fungus causing the eggplant blight, *Phyllosticta hortorum*. The oval Phyllosticta spores and the long narrow Phlyctaena spores developed in culture. Cultures of the Gnomonia and cultures of *Phyllosticta hortorum* cannot be told apart. Inoculation experiments, however, have always been negative. While it may be that the Gnomonia has no connection with the Phyllosticta, it is very probable that the two are closely related species.

LITERATURE ON AMERICAN PLANT DISEASES[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY AND FLORENCE P. SMITH, ASSISTANT

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PHYTOPATHOLOGY

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APRIL, 1917

THE PERFECT STAGE OF GLŒOSPORIUM VENETUM

WALTER H. BURKHOLDER

WITH THREE FIGURES IN THE TEXT

During the early summer of 1914 while studying the anthracnose disease of the raspberry at Brant, New York, a peculiar ascomycete was observed by the writer. The fungus although not of general occurrence was found only in the anthracnose lesions (fig. 1), and arose from the stroma of the pathogene *Glœosporium venetum* Speg. This led to the belief that there was a possible connection between the two fungous forms and a number of inoculation experiments were conducted in order to verify this assumption.

It was difficult to obtain ascospores for making inoculations owing to the scarcity of the ascocarps. Furthermore the ascospores were borne in the same lesion with the conidia of *Glœosporium venetum* and a separation of the two types of spores was practically impossible. It was finally decided to use spores from a culture of the fungus developed from a single ascospore.

Several attempts were made to isolate the fungus. The poured plate method first employed was discarded later on account of the difficulty in obtaining ascospores sufficiently removed from the conidia, the latter usually being in great abundance. A second method and similar to one used by Barber[1] was also tried. This consisted in crushing the ascocarps in a drop of sterilized water on a sterilized slide. A glass tube with a bore of about 3 mm. was drawn to a capillary tip at one end; to the opposite end was fastened a piece of rubber tubing about 40 cm. in length. The free end of the rubber tube was placed in the mouth and by manipulating the glass point with the hand, spores could be drawn into the bore of the tube. The great difficulty in using this method with the fungus under consideration was the fact that the ascospores were very gelatinous and had a tendency to adhere to the glass slides, refusing to enter the

[1] Barber, M. A. On heredity in certain microorganisms. Kansas Sci. Bul. 4: 3-48. 1907.

bore of the tube. Single asci, however, could be picked up in this manner and cultures of the fungus were obtained.

A third method and the most satisfactory one employed was made use of after it was observed that the ascospores were forcibly ejected from the asci. The culture used in the following experiments was isolated on August 14, 1914, and in the following manner: A sterilized petri dish containing a thin layer of nutrient agar was inverted over a small piece of raspberry cane which contained the fruiting bodies of the ascomycete. The cane was then moistened and the petri dish allowed to remain in place for about fifteen hours, during which time a few spores were ejected

FIG. 1. ANTHRACNOSE LESIONS ON CANES OF THE BLACK RASPBERRY

from the asci and lodged upon the agar surface above. A single ascospore was then located by means of a low magnifying microscope and the position of the spore marked on the lower side of the petri dish. The germination and development of the spore was observed and when sufficient growth had taken place the fungus was transferred to a test-tube. Growth of the ascomycete in culture is identical with that of *G. venetum*. On potato agar the fungus develops a wrinkled sclerotium-like mass with minute filamentous hyphae radiating in all directions. The colony is at first circular, growing very slowly and varies with age from a pale vinaceous pink to a maroon color. The cells of the mycelium composing the fungous mass vary greatly in size. They are globose and filled with oil

globules and pigments which give color to the fungus. Only on rare occasions and on media containing a small percentage of agar do filamentous hyphae extend for any distance from the sclerotia-like formations. This growth on artificial media although identical with that of *G. venetum* is decidedly different from that of any species of Gloeosporium which has a perfect stage belonging to the genus Glomerella.

As the writer has continually found to be the case with cultures of *G. venetum*, difficulty was encountered in finding conditions favorable for the sporulation of the fungus. It was finally observed, however, that a sudden change in the humidity of the culture tube caused a production of conidia which were obtainable in sufficient numbers for use. In order to effect this change the fungus was grown on three-per-cent potato agar until large sclerotia-like masses were formed. These masses were transferred to sterilized bean pods in tubes which contained several centimeters of water. The cultures were then incubated at a temperature of 24°C. and at the end of three days numerous conidia were produced which were identical with the conidia produced in culture by *G. venetum*. It was also observed that this sporulation was not continuous, but ceased after the first production of spores. Furthermore, a culture of the fungus subjected from the beginning to a moist condition produced no spores or at least, but relatively few. This apparently indicates that the sudden increase in humidity acts as a stimulus to spore formation.

By dropping these fungous masses bearing conidia into a small amount of water the spores readily fall off and can be sprayed over the infection court. The germination of these conidia is fairly rapid, but the percentage of germination is low. In most cases not more than five or ten per cent of the spores germinate.

INOCULATION EXPERIMENTS

Early in the winter of 1914 a number of roots of the Columbian variety of the raspberry were obtained and planted in the greenhouse. Owing to the earliness of the dormant period and to the unfavorable conditions arising within the greenhouse, the plants grew slowly, and gave a very stunted growth. All inoculation experiments with these plants gave negative results. The anthracnose lesions appear only on tender succulent canes and apparently the canes which had developed slowly on the greenhouse plants were too hard for the fungus to infect.

Later, about the first of March, 1915, a few raspberry plants of a red variety were secured which were tender and growing rapidly. On March 4, two canes were sprayed with a suspension of conidia from a culture of the fungus developed from a single ascospore and the canes were covered

with bell-glasses lined with moist filter paper. These glasses were plugged at the top with cotton and allowed to remain over the canes for two days before removing, while two other canes in the same bed remained untreated. On March 20, small purple spots had appeared on one of the canes. These infections grew slowly, much slower than an anthracnose lesion develops under field conditions, but spots typical of those caused by *Glœosporium venetum* were produced. Miscroscopical examination of the spots showed conidia of *G. venetum*.

Again on April 15, four very tender canes of a black-cap variety of raspberry were sprayed with a suspension of conidia as above. Bell glasses were placed over the plants as in the previous experiments and one check plant was used. A sample of the conidia used was placed in a drop of water on a slide, and about eight per cent of the spores germinated. After one week, April 21, a number of small purple spots had appeared on the four canes, and these later developed into typical anthracnose lesions. The check plant remained healthy.

THE DEVELOPMENT OF THE ASCIGEROUS STAGE

From the positive results of the inoculation experiments and also from the examination of the fungus in culture it is evident that the ascomycete under consideration is the perfect stage of *Glœosporium venetum*. The systematic position of the fungus, however, is rather difficult to determine. Its morphology is entirely different from the perfect stage of any species of Glœosporium previously described. This, however, is not surprising as *G. venetum* has always been considered distinct from the other species of that genus.

The ascigerous stage of the fungus was first observed on the hybrid raspberry commonly known as *Rubus neglectus*. Later it was collected in various parts of New York State on the black raspberry (*Rubus occidentalis*) and the American red raspberry (*Rubus idœus* var. *aculeatissimus*). Rees[3] also reports having found it in Washington on the blackberry (*Rubus* sp.).

During the summer following the discovery of the ascigerous stage close observation was kept of the fungus on the young canes to determine when the ascocarps first began to develop. This proved to be about the middle of August. At this time the fruiting bodies which greatly resemble those in the family Myriangiaceæ appear as minute spots, deep brown to black, singly or in groups scattered over the buff-colored and sunken portion of the anthracnose lesion. These spots are barely visible to the eye

[3] Rees, H. L. Experimental spraying for blackberry anthracnose in 1915. Western Washington Exp. Sta. Mo. Bul. 3[8]: 1-10. 1915.

and only so on account of the contrast in color with the surrounding tissue. After passing the winter the entire lesion assumes a dark brown color and then the pustules are observed with great difficulty even with a hand lens.

A careful examination of the diseased area upon which the asco arps are found proves it to be a typical anthracnose lesion. The buff-colored portion is fungous tissue, more or less plectenchymatous in structure. It is composed of very small hyphae which are difficult to distinguish unless carefully stained and it is the same tissue from which arise the conidiophores of the Glœosporium stage. The ascocarps arise from the stroma and are pulvinate structures usually circular in outline, but they frequently coalesce, forming spots of various shapes. They are approximately 0.07 by 0.07 to 0.37 mm. in diameter. The tissue of the ascocarp is more or less pseudoparenchymatous with larger and thinner-walled cells than those of the stromataceous tissue (fig. 2). The outer layer of the ascocarp is composed of thick-walled brown cells which form a shield-shaped structure less perfect, however, than those observed in the family Microtheriaceæ. When the fruiting body is mature the cells of this outer layer split apart in a stellate manner and crumble away. Within the shield the ascocarps are hyaline and contain the asci which are scattered irregularly through the fungous tissue. There is no differentiated cavity for the asci.

The asci were first observed in the immature condition at which time they appeared as globose bodies containing a homogenous mass of protoplasm, and greatly resembling thick-walled oogonia. These asci may lie in contact with each other but frequently they are separated by the fungous tissue. The mature ascus is thick-walled and measures 24 to 30μ in diameter. In a few cases the ascus has appeared to be slightly stalked and attached to the base of the cavity in which it is borne.

In the autumn or more often in the spring the asci mature and the homogenous mass of protoplasm gives place to eight four-celled ascospores. These spores which are borne parallel to each other in the ascus, are hyaline with gelatinous walls, and constricted at the septa. The basal cell is somewhat more obtuse than the apical cell. The mature ascospore measures 18 to 21μ in length by 6.5 to 8μ in diameter.

In the formation of these spores the middle septum is laid down much earlier than the other two, and for this reason it is not uncommon to find two-celled spores. With the division of these cells giving rise to the four-celled condition, the constrictions are not so great as at the first septum. Occasionally one of the cells fails to divide and an ascospore of three cells is formed. During the formation of the spore the disintegration of the fungous tissue about the asci takes place and with the rupturing of the outer layer of the ascocarp the asci are exposed. Frequently the

asci lying in this exposed condition surrounded by the remainder of the ascocarp give the appearance of a true discomycete. This, however, is due to the persistency of the outer cells of the shield-like layer which

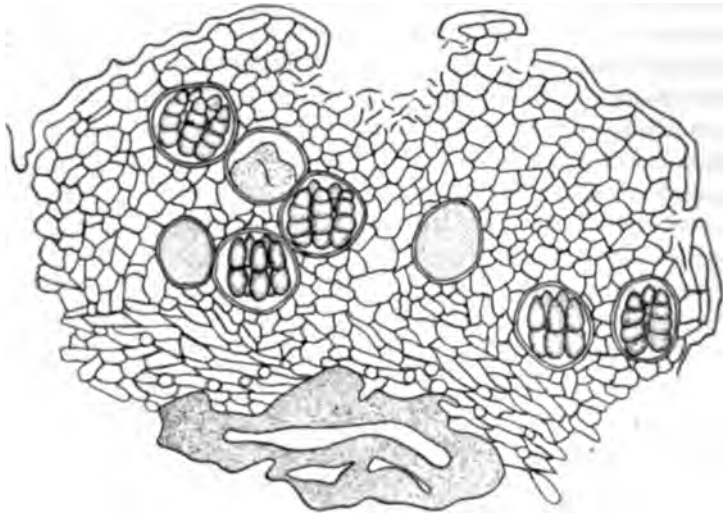

FIG. 2. CROSS-SECTION OF ASCOCARP OF PLECTODISCELLA VENETA
Various stages in the development of the asci are shown

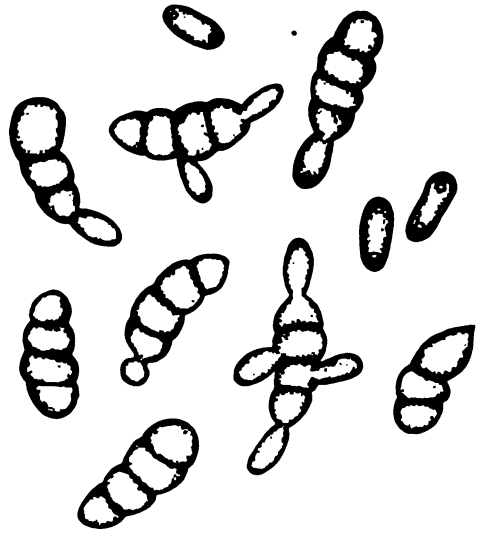

FIG. 3. ASCOSPORES OF PLECTODISCELLA VENETA AND THEIR METHOD OF GERMINATION

covers the immature ascocarps. With the presence of sufficient moisture the exposed asci elongate approximately three times their usual length. This process is very rapid and may be observed under a microscope when a fragment of tissue containing asci is placed in a drop of water. The lower portion of the ascus remains fastened in the cavity in which it was borne, giving a conical shape to the body which raises itself above the surrounding tissue. The spores gather at the tip of the ascus and from there are ejected into the air. They have been caught above the lesions at a distance of one centimeter.

In a single ascocarp all the asci are never in the same state of maturity and the ascocarps also seem to vary in this respect. Mature ascospores were first observed about the first of June while immature spores were present in August.

GERMINATION OF SPORES

When placed in tap, rain or distilled water, or on nutrient agar, the mature ascospores germinate readily (fig. 3). They swell somewhat and within less than two hours a short sterigma is produced from one or each of the cells. A sprout conidium is formed which is oblong to elliptical and identical with the conidia of the fungus. When fully mature the sprout conidia drop from the sterigmata but do not germinate immediately. After a short period of rest, twelve to twenty-four hours, a germ-tube is sent forth and mycelium is formed. When an ascus is placed in a drop of water or on agar the spores within will germinate by sending the sterigmata through the wall of the ascus and produce the sprout conidia on the outside. These, in turn, germinate. After the production of the secondary spores, however, the ascospores shrivel and disintegrate.

SYSTEMATIC POSITION

The morphology of the fungus of the raspberry anthracnose, especially the character of the asci scattered irregularly through a pseudoparenchyma is similar to that of the old family Myriangiaceæ. In a revision of this family some years ago by von Höhnel[3] but five genera out of twenty-three were retained, and since then but one new genus, Ascostralum Sydow[4] has been added. The perfect stage of *Glœosporium venetum*, however, does not appear to fall in any of these genera, nor in any of the genera of closely

[3] Höhnel, F. von. Fragments zur mycologie VI. Mitt. 244. Sitzungsber. M-N Classe, k. k. Akad. Wiss. Wien. **118**: 349-376. 1909.

[4] Sydow, H. von and Sydow P. von. Beschreibungen neuen südafrikanischen Pilze. Ann. Myc. **10**: 41-42. 1912.

related families. More recently Woronichin[5] described a new genus, Plectodiscella, based on a single species which he found occurring on the leaves of the apple and pear. This genus is closely related to Elsinoe of Raciborski[6] but differs mainly in that the stroma is not borne beneath the epidermis. *Plectodiscella Piri*, the representative of the genus, is so similar in morphology to the ascigerous stage of *Glœosporium venetum* Speg. that apparently there is a distinct relation between the two. A brief description of Woronichin's species is here set forth: A more or less imperfect stroma is formed in the epidermal and sub-epidermal cells of the leaf, which is at first sub-cuticular. From this arises a fungous tissue in which are borne irregular globose asci, each containing eight four-celled ascopores. In some instances the asci are separated by the fungous tissue and in others they lie in contact with each other. Woronichin is uncertain as to the nature of this tissue between the asci. He says, "Was für Elemente die zwischen den Ascen befindlichen Zwischenräume ausfüllen, gelang es nicht genau aufzuklären." He does not consider, however, that it is cellular. This is also the first impression received in regard to the raspberry fungus, due to the fact that the cells are minute and disintegrate very early. The cellular structure of the fungus on raspberry was determined only on young material and then after it was fixed and stained. In *Plectodiscella Piri* a shield-like arrangement composed of one layer of dark cells covers each ascocarp and later breaks apart in order that the asci may be exposed. In the perfect stage of *Glœosporium venetum* this is present but is clearly seen only in the immature stages before rupturing occurs. Woronichin does not refer to an imperfect stage for his fungus.

Plectodiscella Piri is considered to be far enough removed from the Myriangiaceæ or any of its closely related families to be placed in a new family. This, Woronichin describes as Plectodiscelleæ and states that it occupies a systematic position somewhere between the Plectascales and the true Discomycetes. Here he places his fungus *P. Piri* but gives no exact characters for his genus. Only the family and species are described.

Taking all characters into consideration, the perfect stage of *Glœosporium venetum* Speg. appears to belong to this genus and therefore the following name is proposed:

[5] Woronichin, N. M. Plectodiscella Piri, der Vertreter einer neuen ascomyceten Gruppe. Mycol. Centralb. 4: 225-233. 1914.

[6] Raciborski, M. Elsinoë Rac. nov. gen. Parasitische Algen und Pilze Java's 1: 15-16. 1900.

Plectodiscella veneta sp. nov.

Stromatibus solitariis vel gregariis, pulvinatis, epidermide fusca discoida, mox dehiscente, intus contextu hyalino, pseudoparenchymatico vel indistincto, plerumque pluriloculigeris, loculis monascis, irregulariter sparsis; ascis globosis, 8 sporis, 24-30μ; sporidiis ovoideo-ellipsoideis, saepe flexis, hyalinio, 3-septatis, constrictis, cellula basilare obtusa, 18-21 x 6.5 x 8μ.

Hab. In ramis caulibusque vivis Rubi occidentalis, R. idaei var. aculeatissimi et R. neglecti. New York, America boreale. Status conidiophorus est Glœosporium venetum Speg.

CORNELL UNIVERSITY
ITHACA, NEW YORK

PUCCINIA SUBNITENS AND ITS AECIAL HOSTS

ELLSWORTH BETHEL

Puccinia subnitens Diet. is a common rust on *Distichlis spicata* from the Atlantic to the Pacific coast. The telial host is especially abundant in the alkaline soils of the desert regions of the western United States. Prior to 1904, *Chenopodium album* was the only known aecial host of this rust. In the summer of 1904, Rev. J. M. Bates, from field observations in Nebraska, concluded that aecia on species of Cleome, Sophia, Lepidium, Erysimum, and Salsola were related to *Puccinia subnitens*. These suggestions were communicated to Dr. J. C. Arthur, who later in the summer succeeded in growing the teliospores on these hosts, thus confirming the deductions made by Bates. Dr. Arthur, in giving the results of these cultures, remarks (Jour. Myc. 11: 50–67), "We have here a demonstration of the remarkable fact, not known for any other species of rust, that *Puccinia subnitens* has aecia growing with equal vigor on three families of plants." Later, Arthur grew it on *Capsella* (Bursa) sp., *Atriplex hastata*, and doubtfully on *Sarcobatus* sp. He has grown this species on nine or ten genera in three families.

For ten years or more, the writer has observed the aecia of this rust abundant in Colorado on species of Polygonum, Chenopodium, Lepidium, Capsella, Cleome, Salsola, and Abronia, and in 1912 made successful cultures on all of these genera except Abronia. During the past summer, aecia which seemed unmistakably related to *Puccinia subnitens* were found on plants of several other genera, and cultures were made to determine the correctness of these observations. Likewise all previous cultures were repeated with the result that the aecia were produced on 22 species in 6 families, and 15 genera. The following is a list of successful cultures.

1. POLYGONACEÆ: *Polygonum aviculare* L., *P. erectum* L., *P. ramosissimum* Michx.

2. CHENOPODIACEÆ: *Salsola pestifer* A. Nels., *Chenopodium album* L., *C. glaucum* L., *C. lanceolatum* Muhl., *C. pagonum* Reich., *Monolepis nuttalliana* (R. & S.) Greene, *Kochia scoparia* (L.) Roth.

3. AMARANTHACEÆ: *Amaranthus retroflexus* L., *A. blitoides* S. Wats.

4. NYCTAGINACEÆ: *Abronia fragrans* Nutt.

5. CRUCIFERÆ: *Capsella Bursa-pastoris* Medik., *Lepidium densiflorum* Schrad., *L. medium* Greene, *Erysimum asperum* DC., *Sophia pinnata*

(Walt.) Britt., *Roripa palustris* (L.) Bess., *Thlaspi arvense* L., *Sisymbrium altissimum* L.

6. CAPPARIDACEÆ: *Cleome serrulata* Pursh.

It will be observed that the above six families constitute two groups. The first four comprise a group of contiguous families, and the last two another group, likewise contiguous but rather remote from the first. *Æcidium fumariacearum* Kell. & Swingle on Corydalis is probably related to *P. subnitens*, though no cultures were made. If this connection should be established it would add another family, Papaveraceæ, contiguous with the second group, a total of seven families.

It is not uncommon to find the aecia in abundance on a half dozen or more host plants at one place, and it manifests only slight racial tendencies, though it seems to infect certain hosts, such as Thlaspi, Kochia, Monolepis, Amaranthus, Roripa, and Erysimum very sparingly. The aecia occur in abundance on plants of all other genera listed above.

Stanleya pinnata (Pursh.) Britt. bears a large orange-red aecium characteristic of *P. subnitens*, however, this host failed to become infected, though seven cultures were made under the same conditions, and with the same material that was used in the successful cultures on other hosts. Cultures were attempted on *Atriplex hastata* L., *A. canescens* James, *A. confertifolia* S. Wats., and *Sarcobatus vermiculatus* (Hook.) Torr. with no results. Arthur has reported successful cultures on *Atriplex hastata* L. with teliospores from Delaware, and on *Sarcobatus vermiculatus* (Hook.) Torr. with teliospores from Nevada. The aecia on the latter host, which closely resemble those of *P. subnitens*, are related chiefly, or entirely, at least in Colorado, to *Puccinia luxuriosa* Syd. on *Sporobolus airoides* Torr., as has been shown by the writer by several successful cultures the past season, both from aeciospores and teliospores, so that there can be no doubt of this relationship. Further, many sowings of teliospores of *P. luxuriosa* on the aecial hosts of *P. subnitens* made through two seasons gave negative results.

Æcidium Abroniæ E. & E. was described on *Abronia* sp. from Fort Collins, Colo. Many cultures both in the field and the garden show that it is the aecial stage of *P. subnitens*. It is common on *Abronia fragrans* Nutt. in Colorado and occasionally collected on *A. elliptica* A. Nels., and *A. micrantha* A. Gray.

Late in the summer cultures were attempted on Beta, Blitum, and Portulaca but no infection resulted, presumably for the reason that the teliospores had probably already germinated. Successful cultures were obtained on either Raphanus, or Brassica, but the plants died before developing sufficiently for determination. The aecium on Cleomella, as noted by Arthur, also is probably related to *P. subnitens*. Cul-

tures will be made again next season on Stanleya, Atriplex, Corydalis, Cleomella, Beta and plants of some other suspected genera, and if these prove to be accial hosts of this rust, which seems very probable, we shall have a grand total of more than a score of genera in seven families—a remarkably large number of accial hosts for a single species of rust.

COLORADO STATE MUSEUM
DENVER, COLORADO

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE WHITE PINE BLISTER RUST

W. A. McCUBBIN

I. MODE OF INFECTION ON THE PINE

Only indefinite references to the method of infection of the pine by *Cronartium ribicola* have appeared in current literature. From these references one gathers the impression that infection takes place through the bark, and probably by way of wounds or abrasions. Having an opportunity for studying a considerable number of pine infections in 1916, some attention was given to this point, and records were made of the origins of cankers where such origins could be determined.

In most cases the determination was not difficult, owing to the fact that in a healthy pine branch the fungus spreads out from the court of entry in a very regular and equal manner, and that its progress is marked by swelling or discoloration or both, or else the cortical tissue is killed in an equally radial fashion. By taking note of this habit one can readily locate the point of original infection in most cases, especially in the earlier stages.

TABLE 1

Records of specific cases to show mode of infection of pines by Cronartium ribicola

LOCALITY	NUMBER OF INFECTIONS	ORIGIN OF LESION		
		Leaf fascicles	Wounds	Undetermined
Secords	177	148	8	21
Four-mile Creek	38	34	1	3
Cookstown	792	743	5	44
Totals	1007	925	14	68

Very early in this study it became apparent that the chief mode of infection was by way of leaf fascicles through the so-called short shoots. In these pines, which were all healthy and which grew in situations where they were fairly free from accidents, wound infection played but a very small part.

According to the tabulated results about 92 per cent of these young blister cankers originate in leaf-bundle infection. This percentage in-

cludes only those cases where the point of origin could be confidently established, but it is highly probable that a large proportion of the number listed as undetermined should also find a place here, and it might not be overstepping the mark to ascribe at least 95 per cent of these blister cankers to leaf fascicle infection.

One may consider that the sporidia from the currant leaves are lodged among the bases of the needles and from this position can then attack the short shoot which bears these leaves. In a number of instances a few of the leaves on such shoots were found to be dead while the rest of those in the fascicle were quite healthy; in other cases all the leaves in the fascicle had been destroyed and often the short shoot and even a small area in the cortex at its base were also killed. In milder cases, especially where the growth of the tree was very vigorous, the fungus did not kill either the leaves or the short shoot, but induced in the latter a pronounced stimulation of growth, so that the short shoot became enlarged and bulbous in appearance.

In these peculiarities of short shoot infection may lie a possible explanation of the year of dormancy which so evidently obtains in a great majority of cases. If, during the summer after infection, the fungus progresses only into the short shoot or slightly beyond it into the adjacent cortex, it would be difficult to recognize these minute symptoms and there would be the so-called dormant year.

II. LIFE CYCLE OF THE FUNGUS ON THE PINE

In general the tendency has been to regard the life of *Cronartium ribicola* on its pine host as more or less indefinite, varying from one to two years up to six years or more; that is, from the time of infection until aecia are produced from one to six years might elapse. In the study of this disease in Ontario in 1916 evidence has come to hand which indicates that the fungus tends to reach the aecial stage in a fairly definite period, but that this normal course of development may be shortened or lengthened because of certain favorable or unfavorable factors.

In the Niagara Peninsula in 1916 there were found a number of young pine infections on trees growing close to black currants, and though these currants have been badly rusted since and including 1914, there is reason to think that no rust was present on them before that season. In any case no sign of infection was visible on these pines in 1915, although they were certainly exposed to infection during the previous year. Moreover, in the summer of 1916 no disease was met with on the growth of 1915 although these pines must have received infection in 1915. On the other hand, these five lots of pines developed 223 infections in 1916, all on the

growth of 1913 and 1914. It seems reasonable to think, therefore, that during the year after infection there are no symptoms of a visible nature in infected pine branches. Additional evidence on this point recently has been obtained from another district (Cookstown, Simcoe County, Ontario), where a young nursery row of white pines was severely infected from black currants growing side by side with them. Out of the 1412 blister cankers recorded from these rows not one was found on the growth of 1915 although the pines were certainly subject to infection in the previous year. On the twig growth of 1914 there occurred some 286 cases, which number gives a strong indication as to the yearly infection that might be expected here.

While the above evidence from these two cases is scarcely conclusive, it is sufficiently extensive and clear-cut to warrant the assumption that in the great majority of cases the season following infection is a "dormant" year.

When the character of all the 509 infections recorded above is examined further another point becomes clear. With one exception all of these cankers were in what might be termed the swelling stage, the cortex being typically swollen into a spindle and usually discolored. If these cankers can be taken to represent the normal course of the disease, and since they include all the infections found on the 1914 twigs of these quite normal trees there seems to be no reason why they should not be so considered,—then the third season of the disease is apparently characterized by the appearance of the first visible symptoms, the swellings just mentioned. And if the formation of aecia from these swellings be assumed to take place during succeeding years a fairly normal life cycle will have been obtained. Summarized it would run thus: first season, infection in summer and autumn; second season, dormant period; third season, swelling stage; fourth season, aecia. There is evidence, however, that in the majority of cases the swelling stage may last for two years before the production of aecia. This evidence has been obtained partly from the Cookstown case already mentioned and partly from the Secord case where 177 swellings were found in 1916 on young pines growing close to black currants. When these 177 swellings were arranged in a curve representing the number of them that had occurred on each year's growth it was found that the apex of the curve came in 1914; that is, there were more infections on the growth made in 1914 than on that of any other year. When a curve was prepared similarly from the data obtained at Cookstown, it was of another type, having the largest number of swellings on the growth of 1913 (fig. 1). The nature of this curve thus suggests very strongly that the swelling stage may ordinarily last two years before the aecia are produced.

It is to be noted that the Cookstown curve represents infections begun in several successive years while the Secord curve contains infections of only one year, that of 1914. An attempt was made to compare the two series of results on a basis of something like equality by supposing that in addition to the infections started in 1914 in the Secord case a similar series of infections had begun in 1913. Assuming that the swellings which would presumably arise from these earlier infections in 1915, would still remain in the same stage during 1916, a curve was then con-

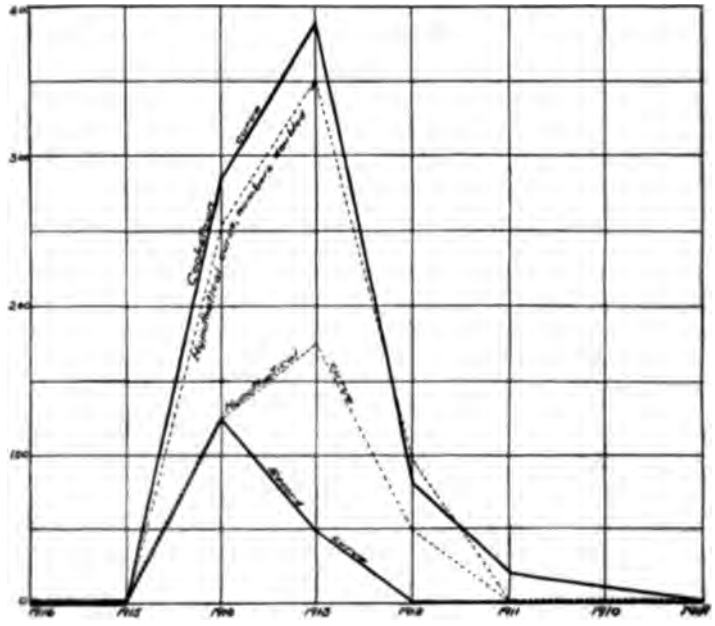

FIG. 1. GRAPH SHOWING NUMBER OF INFECTIONS IN SWELLING STAGE ON THE GROWTH OF EACH INDIVIDUAL YEAR

For explanation see text

structed which would include this hypothetical series of cankers along with those actually present. Such a curve would contain the infections of two successive years and would thus resemble very closely the Cookstown curve. When the numbers in this reconstructed curve were doubled in order to obtain a better comparison with the Cookstown curve, it was seen that except for minor irregularities the two curves are practically identical.

The close concordance of this supposed case with the results of the actual survey provides a striking confirmation of the indication already

given in the Cookstown curve of the continuance of the swelling stage for two years.

The evidence may be presented in another way, by following the course of the disease year by year in a number of shoots of 1911, exposed as in the Cookstown case to a more or less constant annual infection from currants. The number of infections started in these pine twigs in 1911 may be represented by X, those started in 1912 by Y, and those in 1913 by Z. Later infections may no doubt take place in these shoots, but since the number of such infections is known from other considerations to be very small, they may be neglected for the purpose in view. A small letter

TABLE 2

Probable development of the blister rust in pine branches based on a four-years cycle

YEAR	YEARLY PROGRESS OF DISEASE	NUMBER OF SWELLINGS PRESENT EACH YEAR	ESTIMATED PROPORTIONS BASED ON SECORD CASE	COOKSTOWN SURVEY FIGURES
1911	X^i	0	0	0
1912	X^d+Y^i	0	0	0
1913	$X^s+Y^d+Z^i$	X	300	286
1914	$X^b+Y^s+Z^d$	Y	100	390
1915	$X^b+Y^b+Z^s$	Z	10	83
1916	$X^b+Y^b+Z^b$	0	0	22

TABLE 3

Probable development of white pine blister rust in pine branches based on a five-years cycle

YEAR	YEARLY PROGRESS OF DISEASE	NUMBER OF SWELLINGS PRESENT EACH YEAR	ESTIMATED PROPORTIONS BASED ON SECORD CASE	COOKSTOWN SURVEY FIGURES
1911	X^i	0	0	0
1912	X^d+Y^i	0	0	0
1913	$X^s+Y^d+Z^i$	X	300	286
1914	$X^s+Y^s+Z^d$	X+Y	400	390
1915	$X^b+Y^s+Z^s$	Y+Z	110	83
1916	$X^b+Y^b+Z^s$	Z	10	22

attached to each of these symbols conveniently indicates the stage of development, as: *i*, infection year; *d*, dormant year; *s*, swelling stage; *b*, blister or aecial stage.

Using these symbols the accompanying tables have been constructed showing the development of the cankers on these twigs. The first table is based on a four-years cycle, where the swelling stage lasts but one year before aecia are formed, while the second table is based on a five-years cycle, where the swelling stage is continued for two years. In the third column of both tables are entered the number of swellings which will

appear each year, expressed by the symbols adopted. An attempt has been made in the fourth column to substitute values for *X*, *Y*, and *Z*, based on the proportions obtaining in the Secord case. In the Second series the infections begun in 1914 on the growth of 1914, 1913 and 1912 were 127, 49, 0, corresponding to *X*, *Y*, and *Z*, respectively. Adopting 300 as an arbitrary value for *X*, then *Y* becomes approximately 100. *Z* should then be zero, but since there is good reason to think that infections sometimes occur on shoots of three years' standing, though none were found in this case, a nominal value of 10 has been assigned for *Z*.

Beside these estimated proportions in the adjoining column are placed the actual figures of the Cookstown survey. Since these figures indicate the swellings noted at one time on several successive years of growth, they may be used fairly to represent the swellings that would arise on one year's growth in a number of successive seasons. It will be seen at a glance that while the estimated proportions as obtained from the figures of the Secord case do not agree at all with the actual survey figures in the table showing a four-years cycle, there is a strong resemblance between these same two columns in the table where a five-years cycle is used.

It is probable, therefore, that the table giving a five-years cycle expresses more nearly the actual development of the disease than the table giving a four-years cycle; in other words, the normal blister infection on these young pines passes two years in the swelling stage, and the development of the disease as a whole follows this course: First season, infection; second season, dormant period; third season, swelling stage; fourth season, swelling stage; fifth and following seasons, aecia.

The actual time elapsing between infection and the first production of blisters according to this plan of development is something short of four years, but since the course of the disease involves five seasons it seems better for practical reasons to designate it as a five-years cycle.

The above conclusions as to the course of development of the disease on young pines should be fairly trustworthy inasmuch as they are based on a considerable number of blister cankers. On the other hand, there is certain evidence in both the Cookstown and the Secord cases which indicates that precocious or delayed development may occur, and that while the life-cycle outlined may obtain in the majority of cases, it is by no means to be regarded as an invariable rule.

DIVISION OF BOTANY
DEPARTMENT OF AGRICULTURE
OTTAWA, CANADA

SPECIES OF MELAMPSORA OCCURRING UPON EUPHORBIA IN NORTH AMERICA

E. B. MAINS

No species of Melampsora on Euphorbia was known to occur in the Western Hemisphere until the present year. Collections, however, have been made recently in the United States both upon introduced and native species of Euphorbia. These collections, which Dr. J. C. Arthur has kindly turned over to me for study, consist of one collection upon *Euphorbia commutata* Engelm. from Indiana, four upon *E. robusta* Small from Colorado and Wyoming, and one upon *E. Cyparissias* L. from Maine. Of these, it appears certain that the one on *E. Cyparissias* is introduced and those upon *E. robusta* and *E. commutata* are native.

Up to the present time, six Old-World species of Melampsora upon Euphorbia have been recognized, these being *M. Gelmii* Bres., *M. Euphorbiæ-dulcis* Otth, *M. Euphorbiæ-Gerardianæ* W. Müller, *M. Helioscopiæ* (Pers.) Wint., *M. Euphorbiæ* (Schub.) Cast. and *M. Euphorbiæ-Engleri* P. Henn. all of which have very similar uredinia and urediniospores, the separation being by telia and teliospores. Of these the first three are well-marked and distinct species. Of the last three, *M. Helioscopiæ* and *M. Euphorbiæ* while distinguished from the rest by well-marked characters, are separated from each other, according to Müller (1907) only by a rather small difference in the length of the teliospores. This difference may be a real one but is scarcely apparent in such European exsiccati of the two rusts as the author has at his disposal for examination. The last, *M. Eurphorbiæ-Engleri* is a species of doubtful validity. It was set apart by Hennings, owing to its habit of maintaining itself wholly by the uredinia, other spore forms never having been found. This is only known on one species of host.

A study of the North American collections of 1916 shows that they can be readily placed in three species, two of which correspond to two of the above and one of which appears to be undescribed. The Maine collection, which is upon *Euphorbia Cyparissias*, has teliospores which in length and uniform thickness of wall agree very well with *M. Euphorbiæ* upon the same host in Europe, while the Indiana collection upon *E. commutata* with its 31–58μ long and apically thickened (3–6μ) teliospores agrees very well with European material of *M. Euphorbiæ-Gerardianæ*,

the urediniospores of all being similar. The collections upon *Euphorbia robusta* from Colorado and Wyoming, however, are distinguished from all other species of Melampsora upon Euphorbia by certain characters of the uredinia and urediniospores and are considered by the writer as belonging to an undescribed species.

The North American species of Melampsora upon Euphorbia may be keyed out as follows:

Urediniospores 16–23 μ long, paraphyses numerous
  Teliospores decidedly thickened at the apex.....1 *M. Euphorbiæ-Gerardianæ.*
  Teliospores not or only slightly thickened at the apex.....2 *M. Euphorbiæ.*
Urediniospores 16–29 μ long, paraphyses few..3 *M. monticola.*

1. *Melampsora Euphorbiæ-Gerardianæ* W. Müller, Centr. Bakt. 17²: 210. 1906.

O and I. Pycnia and aecia unknown.

II. Uredinia amphigenous and caulicolous, scattered, circular, 0.2–0.5 mm. in diameter, subepidermal, soon naked, pulverulent, pulvinate due to the crowded paraphyses, pale yellow, ruptured epidermis inconspicuous; paraphyses numerous, intermixed with the spores, capitate, 16–19 by 51 58μ, the wall colorless, 2–3μ thick; urediniospores globoid to ellipsoid, 13 16 by 16–20μ; wall colorless, 2–3μ thick, finely and closely echinulate, the pores obscure.

III. Telia caulicolous, probably also amphigenous, circinating about the uredinia, oblong, 0.2 1 mm. long, subepidermal, slightly elevated, blackish-brown; teliospores prismatic, 9–15 by 31–60μ, rounded at both ends; wall light chestnut-brown, darker towards the apex, 1.5μ thick, 3–6μ at the apex.

ON EUPHORBIACEÆ

Euphorbia commutata Engelm., West side of High Lake, Noble Co., Indiana, June 11, 1916, II, III, *C.C. Deam 20083A*, communicated by G. N. Hoffer.

This collection, which is the first collection of a Melampsora upon Euphorbia to be reported for North America, has a range in the length of the teliospore somewhat less than that given by Müller (1907, p. 641) for *M. Euphorbiæ-Gerardianæ* in Europe. A comparison with European material upon *E. falcata* (Sydow Ured. no. 1687) shows a very close agreement, however, both as to the urediniospores and teliospores. The teliospores are not quite so generally thickened at the apex as in the European specimen but still are very decidedly thickened, up to 3–6μ, while the uredinia have the usual pulvinate appearance and abundant paraphyses.

Pycnia and aecia are not known for this species but will doubtless be found upon the same host, since *M. Helioscopiæ*, *M. Euphorbiæ* and *M*

Euphorbiæ-dulcis, the three species of Melamspora upon Euphorbia whose pycnial and aecial stages are known, are autoecious.

2. *Melampsora Euphorbiæ* (Schub.) Cast. Observ. Myc. **2**: 18. 1843.
Uredo Euphorbiæ-Helioscopiæ Pers. *β Euphorbiæ-exiguæ* Pers. Syn. Fung. 215. 1801.
Xyloma (*Placuntium*) *Euphorbiæ* Schubert *in* H. Ficinus Flora der Gegend um Dresden **2**: 310. 1823.
Uromyces verrucipes Vuill. Bull. Soc. Bot France **41**: 285. 1894.
Melampsora Euphorbiæ-exiguæ W. Müller, Centr. Bakt. **17**[2]: 210. 1906
Melampsora Euphorbiæ-Pepli W. Müller, Centr. Bakt. **17**[2]: 210. 1906.
Melampsora Euphorbiæ-Cyparissiæ W. Müller,. Centr. Bakt. **19**[2]: 453. 1907.
Melampsora Cyparissiæ W. Müller, Centr. Bakt. **19**[2]: 561. 1907.
O.[1] Pycnia flattened hemispherical; ostiolar filaments none.
I. Aecia foliicolous and caulicolous, circular to oblong, 0.2–0.5 mm. in diameter on the leaves, 1–4 mm. long on the stems, orange-yellow, without peridium or paraphyses; aeciospores spherical to ellipsoid, 19–24 by 21–28μ; wall closely verrucose.
II. Uredinia amphigenous and caulicolous, scattered, circular or oval, 0.1–0.3 mm. long, early naked, pulverulent, pulvinate from the mass of paraphyses, golden-yellow fading to white, ruptured epidermis inconspicuous; paraphyses intermixed with the spores, numerous, capitate, 16–20 by 31–51μ; wall colorless, 3–4μ thick, smooth; urediniospores globoid to ellipsoid, 13–19 by 17–23μ; wall colorless, 2–3μ thick, closely and finely echinulate, the pores obscure.
III. Telia amphigenous and occasionally caulicolous, scattered, circular to oval, small, 0.1–0.2 mm. long, covered with the epidermis, compact, pulvinate, dark chocolate-brown; teliospores prismatic, 7–13 by 32–45μ; wall chestnut-brown above, lighter below, 1–1.5μ thick, not thickened at the apex, smooth.

ON EUPHORBIACEÆ

Euphorbia Cyparissias L., Bank near Turner graveyard, Isle au Haut, Maine. Sept. 13, 1916, II, iii, *J. C. Arthur*.

3. **Melampsora monticola** sp. nov.
O and I. Pycnia and aecia unknown.
II. Uredinia amphigenous and caulicolous, scattered or in circular groups, circular or oblong, 0.2–2 mm. long, subepidermal, long covered by the epidermis, pulverulent, orange-yellow, ruptured epidermis conspicuous; paraphyses few, intermixed with the spores, capitate, 13–21 by 32–58μ, the wall colorless, 1.5–3μ thick, smooth, the stipe solid; urediniospores globoid, ellipsoid or obovoid, 13–20 by 16–29μ; wall colorless, 1.5–3μ thick, finely and closely echinulate, the pores obscure.
III. Telia amphigenous and caulicolous, circinating about the uredinia, circular or oblong, 0.1–1 mm. long, subepidermal, slightly elevated, blackish-brown; teliospores prismatic, 9–16 by 27–56μ, rounded at both ends;

[1] Description of pycnia and aecia adapted from Dietel (1895).

wall light chestnut-brown below, darker towards the apex, 1.5–2μ thick, 2–4μ at the apex.

ON EUPHORBIACEÆ

Euphorbia robusta Small, Carpenter, Wyoming, Aug. 18, 1916, II, iii, *E. T. & E. Bartholomew 6067* (*Uromyces Tranzschelii* Sydow, 0, III, also present); Colorado Springs, Colorado, plains 10 miles east of city, Aug. 31, 1916, II, iii, *E. Bartholomew, 6104* (type); Palmer Lake, Colo., Sept. 23, 1916, II, III, *E. Bethel* (two collections).

In the younger uredinia, especially, apparently thinner walled urediniospores are often seen mixed with the thicker. After treatment with lactic acid, which serves to differentiate the wall from the cell contents and make it stand out more clearly, the apparent difference is not to be observed.

This species is distinct from other species of Melampsora on Euphorbia. The uredinia are abundant, large, and long covered by the epidermis and since they contain but few paraphyses, after the rupture of the covering epidermis and the escape of the pulverulent mass of spores, they possess a flattened appearance when contrasted with the pulvinate mass of paraphyses of other species on Euphorbia. The urediniospores are much more variable and larger in size than those of the other species.

No pycnia or aecia were found upon any of the collections. They are, however, to be looked for upon the same host earlier in the season since the rusts of this group whose life cycle are known are autoecious with all spore forms, although Jacky (1899) working with *M. Euphorbiæ* on *E Cyparissias* and Müller (1907 p. 449) working with the same rust on *E Peplus* claim to have obtained uredinia by infection from teliospores, yet Dietel (1895) working with *M. Euphorbiæ* on *E. Cyparissias*, and Müller (1907) with the same rust on *E. exigua* on the other hand have shown that pycnia and aecia are produced upon these hosts from infections with the teliospores. Dietel (1889) has also shown that *M. Euphorbiæ-dulcis* Schroet. has pycnia and aecia. Since these stages are developed sparingly, it is probable that the first workers overlooked them and that *M. Euphorbiæ* has all spore forms. Although all the North American collections were examined for pycnia associated with the uredinia none were found, and it is probable that all of these rusts will be found to be autoecious and have all spore forms.

It is interesting to note that from a region in which this group has hitherto not been known so many collections from such widely separated areas should have all been made in one season. It is not so surprising that *M. Euphorbiæ* should be found upon *E. Cyparissias*, as the latter has been brought into this country from Europe and it is likely that the rust was introduced with it. It is, however, surprising that the rust has not previously been found since the host is rather widely distributed.

The other two species of Melampsora are upon native species of Euphorbia and of these *M. monticola* is evidently a purely American species found upon a common western Euphorbia.[2] With its abundant, large, orange-yellow uredinia, it is remarkable that this very striking rust has not been collected before. The other species, *M. Euphorbiae-Gerardianae*, is probably native to this country as well as to Europe, since its European hosts, *E. Gerardiana* and *E. falcata* are not known in this country. Another argument in favor of this assumption is that the species of Melampsora on Euphorbia have in most cases physiological races limited to one species of host as Müller (1906, 1907) has shown. Consequently even if European hosts were found, it would be doubtful if rusts on American species could be considered as having an European origin.

The writer wishes to express his deep appreciation to Dr. J. C. Arthur and Prof. H. S. Jackson for the helpful suggestions and criticism received in the preparation of this paper.

PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

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[2] In a communication recently received from Mr. E. Bethel he says, "The one [*M. monticola*] we have here is undoubtedly native as I found it in the high mountains (above 9000 feet) at Nederland, Colo."

RECENT CULTURES OF FOREST TREE RUSTS

JAMES R. WEIR AND ERNEST E. HUBERT

The determination of various species of rusts found on forest trees of the general region of Montana is a difficult task when descriptive evidence and spore measurements are used. In most cases involving heteroecious rusts successful inoculation is considered very necessary in determining the identity of the species under consideration. Consequently, as a beginning, an attempt was made early in March, 1916, to secure various forms of hypertrophy formed by the bark-inhabiting Peridermia. After collecting, these were placed in the laboratory in large test-tubes with sufficient water to supply the branch or twig supporting the infection. Many needles were always left on the branches or twigs. In this manner the fungus in many of the infections was induced to produce spores prematurely and these were available for culture work at an early date. This process also served to develop successfully the pycnial stage of *Cronartium coleosporoides* (D. & H.) Arth. and *Cronartium Comptoniæ* Arth. which stage preceded the aecial stage in both cases. In the search for material a foliicolous rust on the needles of *Larix occidentalis* was collected for the first time in June, 1916, at various points in Montana and Idaho. The rust was very abundant and widespread. All the caulicolous forms of rusts on forest trees in this region and in the states of Michigan and Minnesota as well as a number of the foliicolous forms were tried on a variety of suspected hosts. All inoculations were isolated at the greenhouse at Missoula, Montana, by the use of celluloid cylinders and cotton plugs. The inoculated plants were sprayed daily with tap water for a period of three to five days following inoculation. The following is a summary of the cultures to date since the last report.[1]

Five plants of *Castilleja angustifolia*, two on May 14, 1916, and three on May 8, 1916, were dusted with newly developed aeciospores (forced in laboratory) of *Cronartium coleosporoides* (D. & H.) Arth. (*P. stalactiforme* type) on *Pinus contorta* from Hayden Lake, Idaho. Of the first two plants one developed uredinia on May 26 and telia on May 29, the remaining plant dying before May 24. Of the other three plants, two

[1] Weir, J. R., and Hubert, E. E. Successful inoculations of Larix occidentalis and Larix europea with Melampsora bigelowii. Phytopath. 6: 372-373. Ag. 1916.

Weir, J. R. and Hubert, E. E. A successful inoculation of Abies lasiocarpa with Pucciniastrum pustulatum. Phytopath. 6: 373. Ag. 1916.

developed uredinia on May 30, followed by abundant telia on June 3. The remaining plant wilted. Four control plants remained normal. This checks the cultures of 1915.[2]

Three plants of *Castilleja angustifolia* were dusted with aeciospores of *Cronartium coleosporoides* (typical gall form) on *Pinus contorta* from Sylvanite, Montana, June 23, 1916. Uredinia were not observed but on July 17, 1916, telia appeared uniformly on all three of the trial hosts. Three control plants remained normal. A similar result was obtained on Castilleja with aeciospores from the gall type on *Pinus contorta* from Evaro, Montana. Telia were recorded July 17, 1916, on one of three trial-hosts. The remaining plants died. Three control plants remained normal.

On May 23, 1916, aeciospores of *Cronartium coleosporoides* (blister type) on young seedlings of *Pinus ponderosa* from Haugan, Montana, were dusted on three plants of *Castilleja angustifolia*. Heavy infections on two and a light infection on one with uredinia on June 5 and telia on June 8 were recorded. Three control plants remained normal. This result also checks similar cultures made in 1915.[3]

On June 21, 1916, aeciospores of *Cronartium coleosporoides* (typical gall form) on 18-years-old trees of *Pinus ponderosa* from Sylvanite, Montana, were dusted on three plants of *Castilleja angustifolia*. The appearance of the uredinia was not recorded owing to absence from the laboratory but on July 17, 1916, telia were noted in abundance on two of the inoculated plants. The other trial-host died. Three control plants remained normal.

The results of the season together with those of 1915 demonstrate that the various caulicolous forms of rusts occurring on *Pinus contorta* and *P. ponderosa* in the Rocky Mountain region are the aecial stage of *Cronartium coleosporoides*. It is not only possible but very probable that the same forms on *Pinus contorta* and *P. ponderosa* as known elsewhere in the western United States belong here also. An examination of the aeciospores from galls on *Pinus contorta*, *P. ponderosa*, *P. attenuata*, *P. coulteri*, and *P. jeffreyi*, from widely separate regions has not brought out any specific characters different from that of the material used in the successful cultures on Castilleja. Attempts were made to infect young leaves of *Quercus rubra* with aeciospores from galls on *Pinus contorta* and *P. ponderosa* but without success.

Aeciospores of *Cronartium Comptoniæ* Arth. on *Pinus banksiana* from Cass Lake, Minnesota, were sown on trial-hosts as follows: On two plants of *Castilleja angustifolia* on May 17, 1916, with negative results; on one

[2] Weir, J. R., and Hubert, E. E. A serious disease in forest nurseries caused by Peridermium filamentosum. Jour. Agr. Research 6: 781-785. Ja. 24, 1916.

[3] Jour. Agr. Research 6: 781-785. 24 Ja. 1916.

plant of *Quercus rubra* on May 18, 1916, with negative results; on one plant of *Comptonia asplenifolia* on May 18, 1916, with positive results, uredinia appearing on June 9 and telia on June 17. All control plants remained normal.

Aeciospores of *Cronartium Comptoniæ* Arth. on *Pinus banksiana* from East Tawas, Michigan, were sown on trial-hosts as follows: On three plants of *Castilleja miniata* on May 24, 1916, with negative results; on one plant of *Quercus rubra* on May 24, 1916, with negative results; on two plants of *Comptonia asplenifolia* on May 23, 1916, and June 13, 1916, respectively. Uredinia were noted on June 13 and telia on June 23 on the first plant and uredinia on June 30 on the second. Two control plants remained normal. Aeciospores were sown on one plant of *Myrica carolinensis* and one of *Myrica gale* on May 26, 1916. Uredinia on June 13 and telia on June 25 were noted on both plants. Two control plants remained normal.

A collection of leaves of the previous season (1915) of *Populus tremuloides* bearing telial sori of *Melampsora medusæ* Thüm. was made in Pattee Canyon two miles southeast of Missoula, Montana, on March 12, 1916. A portion of these over-wintered leaves were placed in moist chambers on March 15, 1916, and a few days later upon examination of the yellowish-brown, downy layer formed on the sori, it was found that sporidia were present. These fresh sporidia were used to inoculate newly formed needles of *Larix occidentalis*. Two separate inoculations were made on March 22, 1916, followed by two others on March 26, 1916. Individual branches of trees of *L. occidentalis* were used for the test. On April 3, the first inoculation had resulted in pycnia which were accompanied by small drops of hyaline liquid. On April 4 the aecia were present on the under side of the needles. The second inoculation gave pycnia and aecia on April 4, the third and fourth inoculations resulting in pycnia and aecia on April 8. The four control plants of *L. occidentalis* as well as the needles on the inoculated trees which were not included within the cylinders remained normal. All the needles subjected to the inoculation became infected and developed a large number of sori of both stages (O and I) of the rust.

On April 9, 1916, germinating teliosporic material of *Melampsora medusæ* Thüm. on *Populus tremuloides* was sown on two small trees of *Larix europea*. On April 26, an abundant development of pycnia were recorded and on May 1 the aecia appeared. The pycnia and aecia and their respective spore-forms on the two species of larch were found upon examination to be identical. The telial material failed to infect *Tsuga heterophylla* and *Pseudotsuga taxifolia*. The control plants in all cases remained normal.

Teliospores bearing sporidia of *Melampsora medusæ* Thüm. on leaves of *Populus trichocarpa* were collected at Haugan, Montana, on June 5, 1916.

On June 7, these were sown on one small tree of *Larix europea*, two of *L. occidentalis*, one each of *Tsuga heterophylla*, *T. caroliniana*, and *Pseudotsuga taxifolia*. On June 15, pycnia appeared on the needles of *L. europea* and on the needles of the two trees of *L. occidentalis*. Aecia developed on *L. europea* in abundance on June 24 and on *L. occidentalis* on June 22. Negative results were secured on *Tsuga heterophylla*, *T. caroliniana*, and *Pseudotsuga taxifolia*. Control plants remained normal.

Teliospores bearing sporidia of *Melampsora bigelowii* Thüm. on *Salix cordata mackensiana* from Deborgia, Montana, were sown on *Larix occidentalis* and *L. laricina* on June 7, 1916. One small tree of each species was used. Pycnia appeared on June 15 and aecia on June 18 on the needles of *L. occidentalis*. On *L. laricina*, the pycnia were noted on June 15 and the aecia on June 18. A heavy infection occurred. Control plants remained normal.

Aeciospores of *Pucciniastrum pustulatum* (Pers.) Diet. on *Abies lasiocarpa* resulting from an inoculation with the telial stage on *Epilobium angustifolium*[4] were sown on two plants of *Epilobium angustifolium* on May 18, 1916. Uredinia developed on June 4, on the underside of a majority of the leaves subjected to the spores. Only the younger leaves became infected. Control plants remained normal. This completes the cycle for *Pucciniastrum pustulatum*.

Teliospores of *Gymnosporangium tubulatum* Kern on *Juniperus scopulorum* from Missoula, Montana, were sown on two plants of *Cratægus douglasii* on April 27, 1916. Pycnia in abundance appeared on May 8 and 11 and aecia on May 22 and 24. Control plants remained normal. This checks a previous culture.[5]

Teliospores of *Gymnosporangium nelsoni* Arth. on *Juniperus communis* from Bonner, Montana, were sown on two plants of *Amelanchier alnifolia* on May 21, 1916. On June 9, pycnia appeared in abundance, the aecia developing on June 28. Control plants remained normal.

Teliospores of *Gymnosporangium nelsoni* Arth. on *Juniperus scopulorum* from Bonner, Montana, were sown on two plants of *Amelanchier alnifolia* on May 21, 1916. Pycnia appeared on June 6; aecia were noted to be developing on June 28. Control plants remained normal

Several of the cultures are still in progress of development and as soon as results on these are secured further information on the rusts of forest trees in this region will be available.

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[4] Phytopath. 6: 373. Ag. 1916.

[5] Weir, J. R. Telial stage of Gymnosporangium tubulatum on Juniperus scopulorum. Phytopath. 5: 218. Ag. 1915.

A RHIZOCTONIA OF THE FIG

J. Matz

With Plate II and Three Figures in the Text

A fungus possessing characteristics common to Rhizoctonia was observed to occur in the years 1915 and 1916 on leaves, twigs and fruit of the fig, *Ficus carica*, at Gainesville, Florida. Superficial, silvery to yellowish white, thin mycelial strands of this fungus can be seen to radiate loosely from yellow to dirty brown infection centers in the fig leaves and spread over the surrounding green tissue. Later as more of the green tissue becomes discolored by the invading mycelium, the upper surfaces of these enlarged brownish areas become silvery white and dry, the mycelial strands become less, if at all visible, while the under surfaces at the corresponding points remain light brown to brown and are usually covered with a visible web of mycelium (fig. 1). Small, immature white, as well as mature brown to dark brown sclerotia attached to mycelial threads are often found on the petioles and midribs of infected leaves, but seldom are sclerotia found on the blades of such leaves. Loosely woven, silky strands of the mycelium of this fungus may be traced to some distance on the twigs. More or less dense accumulations of sclerotia are usually grouped on one side of the twig (plate II, fig. 2). No direct injurious effect was observed to have been caused to the twigs by the fungus. The fruit of the fig may become more or less covered by the spreading mycelium. Numerous sclerotia are then produced which cling by means of mycelial threads to the fruit and its stalk (plate II, fig. 1).

Portions of diseased fig leaves, and parts of twigs upon which mycelium and sclerotia were plainly visible, were killed and fixed in Carnoy's fluid and embedded in paraffin. Cross-sections of the leaf tissue reveal hyphae penetrating through the stomata into the parenchyma (plate II, fig. 4), while cross and longitudinal sections of the twig tissues do not show the presence of a penetrating mycelium.

This fungus was isolated from the diseased tissues of fig leaves, where mycelium but no sclerotia were present; and from single sclerotia taken from twigs. The pure cultures from both sources were alike in every respect. Pure cultures of this fungus were grown easily for over eighteen months on standard nutrient agar, corn meal agar, and sterilized green

bean pods. In culture this fungus does not differ markedly from its appearance on the host.

This fungus was proved to be the cause of a leaf blight of the fig[1] by repeated infection of healthy fig trees with pure cultures, with the production of typical lesions and by reisolation of the organism.

No spores of any kind were found to be produced by this fungus. Besides being sterile, the fungus has sclerotia connected with fibrils and thus should belong to the genus Rhizoctonia (plate II, figs. 5 and 6). Moreover, its mycelium is in all respects precisely of the same type as that of the other well-established species of this genus, such as *R. Solani* Kuhn and *R. crocorum* (Pers.) DC (fig. 3).

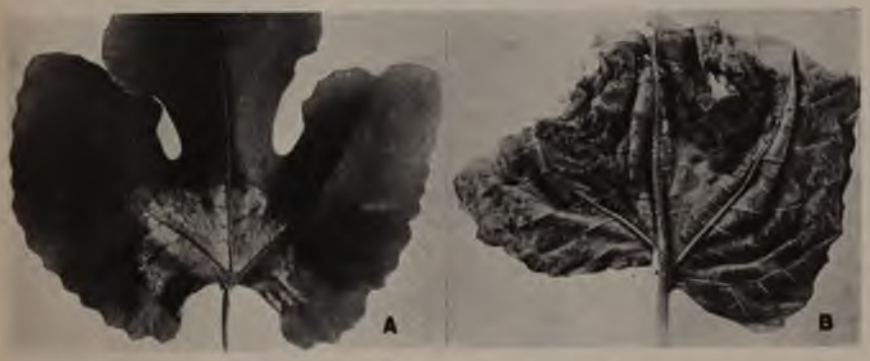

FIG. I. FIG LEAVES ATTACKED BY RHIZOCTONIA MICROSCLEROTIA

A, Upper surface of leaf showing silvery white, dry area and mycelial strands on the surrounding green tissue. × 0.5. *B*, Under surface of diseased leaf. × 0.5.

So far this fungus has not been definitely connected with a perfect stage, although in August of 1916 a few scattered and not fully developed specimens of a Corticium was found in association with the typical sclerotia and mycelium of this Rhizoctonia, on a fig tree which was inoculated the year before with a pure culture of the latter. According to Dr. E. A. Burt's advice, apparently this Corticium is different from *C. vagum* B. & C. and is better referable to *C. Salmonicolor*, Br. & Brs. Several attempts to germinate the spores of this Corticium by plate dilutions and by the method employed by Rolfs[2] to germinate the spores of *C. vagum*, has not proven successful.

[1] An account of the relation of this fungus to the leaf blight of the fig is given in the Florida Experiment Station Annual Report for 1915–1916, to be published in the near future.

[2] Rolfs, F. M. Potato failures. Colorado Agr. Exp. Sta. Bul. 91: 1–33, 5 pls. 1904.

FIG. 2. BLIGHTED FIG FOLIAGE DUE TO RHIZOCTONIA MICROSCLEROTIA

Numerous sclerotia are adhering, by means of slender mycelial threads, to the petioles, stems of fruit and branch. × 0.5.

Of the previously described species of Rhizoctonia the following should be considered here:

Dr. Peltier[3] in a recent publication on parasitic Rhizoctonias in America summarizes as follows: "At the present time there are recognized in America two species of truly parasitic Rhizoctonias; the common form *Rhizoctonia Solani* Kühn (*Corticum vagum* B.& C.), widely distributed and occurring on a great number of hosts; and *R. crocorum* (Pers.) DC., with a limited distribution on alfalfa and potato tubers. A third Rhizoctonia, *Corticum ochraleucum* (Noack) Burt, is found on the leaves of pomaceous fruit trees, while a fourth species isolated from damped-off

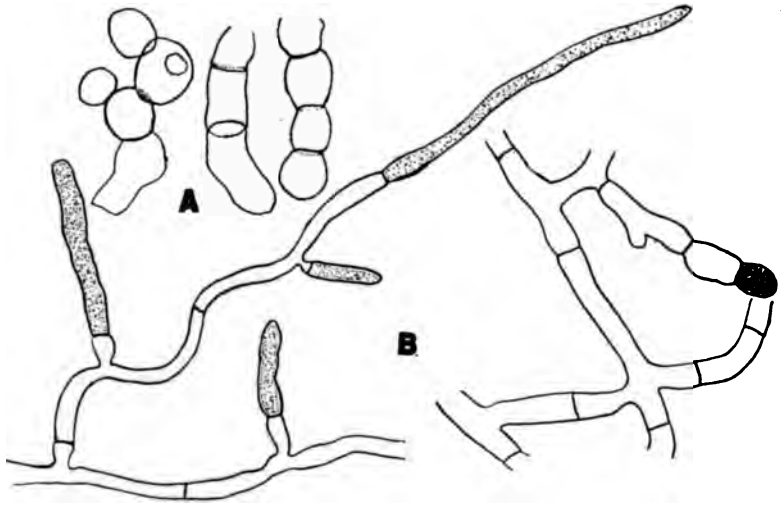

FIG. 3. MYCELIUM OF RHIZOCTONIA MICROSCLEROTIA

A, Short chains and a group of short cells from the margin of young sclerotia. × 333.3. *B*, Vegetative mycelium from a young agar culture on the left, and from an old culture on the right. × 333.3.

onion seedlings is of questionable parasitism." In discussing the growth characters of a number of strains of Rhizoctonia, Peltier[3] makes the following statement (p. 370) regarding the species isolated from damped-off onion seedlings: "The strain from onion produced sclerotia which were entirely different from those of other strains in that they were small (0.5 to 1 millimeter in diameter), perfectly round, bright colored, and developed submerged in the medium." None of these latter characters have been observed in the Rhizoctonia from the fig. The sclerotia of

[3] Peltier, Geo. L. Parasitic Rhizoctonias in America. Illinois Agr. Exp. Sta. Bul. 189:—1916.

this fungus are roundish or oblong, from 0.2 to 0.5 millimeters in diameter, are not bright colored and are produced freely on the surface of but not in the medium in culture.

The effects and manner of attack of *Corticium ochraleucum* (Noack) Burt on pomaceous fruits, as described by Stevens and Hall,[4] are similar to those of the fig Rhizoctonia on its host. However, the sclerotia of the two fungi are entirely different and the perfect stage of the former has not so far been found in connection with the fig Rhizoctonia on fig trees.

Duggar[5] in his recent paper gives sufficient evidence that the common Rhizoctonia in America is *Rhizoctonia Solani* Kühn. This Rhizoctonia has *Corticum vagum* B. & C. as its perfect stage. In comparing the Rhizoctonia of the fig with the *Rhizoctonia Solani* Kühn, obtained from bean seedlings here, the two show pronounced differences on sterilized bean plug and agar slant cultures. On each medium the sclerotia of the first are white at first, turning dark brown with age, and remain small and more or less globose; the sclerotia of the second are white at first, turning light brown to brown or dark brown and are very irregular in size and form (plate II, fig. 6). Mycelium and sclerotia from pure cultures of the fig Rhizoctonia and from *R. Solani* Kühn were placed on moist and growing fig leaves and twigs. Both fungi killed areas in the leaf tissue but *R. Solani* did not produce any sclerotia on the infected parts. Two separate flats of cowpea seedlings were inoculated with the two fungi. *R. Solani* killed 90 per cent of the seedlings, while the fig fungus did not produce any injury to the young plants.

Shaw[6] in his account of a Rhizoctonia which he found on jute, mulberry, cotton, and cowpea, and which he apparently misnames *Rhizoctonia Solani* Kühn, illustrates a fungus which is similar to the Rhizoctonia of the fig in several respects, i.e., the normal occurrence of its numerous, comparatively small, rounded sclerotia on the tips and stems of its respective host plants. However, the diameter of the sclerotia of Shaw's Rhizoctonia is about half of that of the fig Rhizoctonia; the color of the sclerotia of the former is black, while that of the latter is brown to dark brown. Shaw's illustrations (plate IX) indicate a distinct cortex in the sclerotia of his Rhizoctonia, which formation is absent in

[4] Stevens, F. L. and Hall, J. G. Hypocnose of pomaceous fruit, North Carolina Agr. Exp. Sta. Rep. **1908-09**: 32, 76-85, figs. 41-48. 1911.

[5] Duggar, B. M. Rhizoctonia crocorum (Pers.) D. C. and R. Solani Kuhn (Corticium vagum B. & C.) with notes on other species. Ann. Missouri Bot. Gard. **2**: 403-458. 1915.

[6] Shaw, F. J. F. Morphology and parasitism of Rhizoctonia. Mem. Dept. Agr. India **4**: 144. 1912.

The Genus Rhizoctonia in India. Mem. Dept. Agr., India. **7**: no. 4. 1915.

those of the fig. The mode of origin, as described by Shaw, of the sclerotia of the first, has not been observed in the fig fungus.

In culture the sclerotia of the fig fungus develop from dense masses of short hyphae and there the young sclerotia are usually surrounded by short chains and groups of ovoid, short, sometimes elbowed cells.

Zimmerman[7] in describing *Corticum javanicum* on *Coffea arabica*, *Coffea liberica* and several other plants, mentions the occurrence of a sterile mycelium and small (0.15 to 0.3 millimeter in diameter) white sclerotia-like bodies (weisse Kugelen) in association with this Corticum. He states: "dieselben treten sowohl auf der Ober—als auf der Unterseite der Zweige auf und sind ausserdem auch namentlich häufig an den Früchten zu finden;" but no mention is made of its occurrence on leaves. In describing the effects of this fungus on the host, Zimmerman says, "Die unter den Kugelen gelegenen Pflanzenteile sterben—zum mindsten in den nahe der oberfläche gelegenen schichten—ab und erhalten eine dunkelbraune bis schwarze Färbung;" but no direct injurious effect from the sclerotia of the fig fungus was observed on the branches of its host. Regarding the "Kugelen" of Zimmerman's fungus he states: "vertrocknen sie einfach an den zweigen, auf denen sie sitzen." No mention is made here of the change in color which is a dark brown in the sclerotia of the fig fungus at maturity.

Edgerton[8] described a limb blight of the fig, due to *Corticum lætum* Kars. This disease is characterized according to Edgerton's illustrations and descriptions, mainly by the conspicuous fruiting layer of the fungus associated with the diseased parts of the host, but no mention is made of any Rhizoctonia occurring on fig trees afflicted with limb blight.

Kuijper[9] describes a leaf disease of *Coffea arabica* and *Coffea liberica* under the name of "Zilverdraadziekte der Koffie," in Surinam. There is a striking similarity in the character, the manner of attack, and effects of the sterile fungus which causes the Silverdraad disease on *Coffea*, to the Rhizoctonia of leaf blight of the fig. In describing the *Coffea* fungus, Kuijper does not mention nor illustrate anything which approaches a semblance of the dark brown sclerotia which are commonly found in connection with the fig fungus on its host and in pure culture. The sclerotia of the latter are not identical with Kuijper's "hyphenkluwens." These forms occur also in his cultures. He states: "Op plaatsen, waar veel, zijtakken ontstaan strengelen deze zich door elkaar, zoodat op de

[7] Zimmerman, A. Ueber einige an Tropischen Kulturpflanzen beobachtete Pilze I. Centbl. Bact. Abt. II, 7: 102. 1901.

[8] Edgerton, C. W. Louisiana Agr. Exp. Sta. Bul. 126: 13, pl. VII, fig. 1. 1911.

[9] Kuijper, J. De Zilverdraad-ziekte der Koffie in Suriname. Dpt. van den Landbouw, Suriname. Bul. 28:—1912.

bladeren beschreven kluwens ontstaan." In some of his cultures Kuijper obtained "hyphen-opeenhoopingen zoo sterk, dat bijna bolformige lichaampjes van 1 à 2 m.m. doorsnede ontstaan, die bestaanuit een betrekkelijk los hyphenvlechtwerk." Apparently these bodies never become in Kuijper's cultures as compact and colored as do the sclerotia of the fig fungus on various media and on the host.

Brooks and Sharples[10] in their work with *Corticium salmonicolor* B. & Br. (*C. javanicum* Zim) describe four forms in which the fungus appears on rubber trees as follows: "a pink incrustation on the branches or main stem; . . . . white or pale pink pustules arranged more or less in lines parallel with the branches; . . . . part of the fungus on the exterior consists of white or pale pink strands of a cobweb-like texture, which run irregularly downwards over the surface, the strands being sometimes so delicate as to be overlooked; . . . . finally there is the Necator stage. . . . . consists of orange-red (not pink) pustules about one-eighth inch in diameter." In their pure cultures of *C. salmonicolor*, the above authors observed clamp connections in old as well as young cultures, a pink to a bright rose coloration and, "aggregations of hyphae . . . . resembled a number of closely attached Necator pustules." Practically none of these characters were observed in the fig fungus.

From the preceding discussion it follows then that the Rhizoctonia of the fig leaf blight is different from all the true species of Rhizoctonia previously described and thus it should be considered as a new organism which can briefly be described as follows:

Rhizoctonia microsclerotia n. sp.

Sclerotia superficial, small 0.2 to 0.5 millimeters in diameter, white when young, brown to dark brown at maturity, nearly homogenous in structure and color, sub-globose, free from tufted mycelium, not smooth usually single, sometimes conglomerated.

Vegetative hyphae 6 to 8 μ wide, first hyaline and granular, brown and more or less empty with maturity, septate.

Hab. On living leaves, branches and fruit of the cultivated fig, *Ficus carica*, Gainesville, Florida, U. S. A.

[10] Brooks, F. T. and Sharples, A. Pink disease. Dept. of Agri. Federated Malay States Bul. 21: 1-27, fig. 19. 1914.

Rhizoctonia microsclerotia Sp. Nov.

Tuberculis superficialibus, minusculis, 0.2 to 0.5 millimeters in diameter, primum albis deinde fusco-bruneis (intus idem quod extus), similibus fere forma et colore intus ac extus, sub-globosis, floccis myceliaribus deficientibus, non glabris, saepius singulatis, non-nunquam conglomeratia.

Hyphis 6–8 μ, latis, primum hyalinis granulosis deinde bruneosis, septatis.

Hab. in foliis, ramis ac fructibus vivis Fici caricae cultae, Gainesville, Florida, Am. Bor.

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GAINESVILLE, FLORIDA

PLATE II. RHIZOCTONIA MICROSCLEROTIA ON FIG

FIG. 1. R. microsclerotia covering a twig and fruit of the fig. × 1.5.

Fig. 2. Fig twig defoliated by the fungus. Numerous sclerotia are accumulated on one side of the twig. × 1.5.

FIG. 3. Three-weeks-old cultures on sterilized bean pods. R. microsclerotia on left, R. Solani Kühn on right.

FIG. 4. Cross section of diseased fig leaf showing penetration of mycelium through stomata at *a* and *b*.

FIG. 5. Section through a sclerotium of R. microsclerotia.

FIG. 6. Sclerotia from a fig branch. × 25.

MATZ: RHIZOCTONIA OF FIG

BUCKEYE ROT OF TOMATO FRUIT

C. D. Sherbakoff

With Five Figures in the Text

A hitherto undescribed rot of tomato fruit has been under the writer's study since January, 1915, and while there are still many phases of the disease which have to be determined by further work, nevertheless certain important facts have been determined and these findings may be of some use to those who are interested in tomato culture and diseases.

SYMPTOMS

The disease occurs only on the fruit of the tomato on which it appears in the form of a grayish, or pale to dark greenish brown, often distinctly

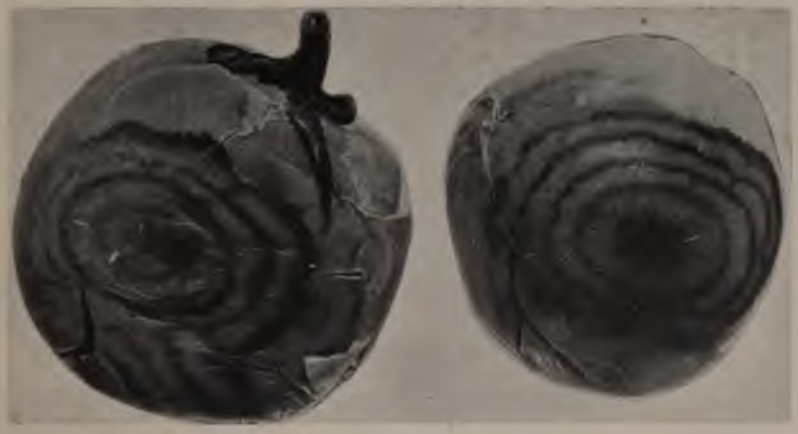

Fig. 1. Buckeye Rot of Tomato

These fruits show the most striking symptoms of the rot. Often the zonation is indistinct and sometimes entirely lacking. These fruits were preserved in formaldehyde for nearly two years. Natural size.

zonate rot (fig. 1). The affected parts of the fruit retain their normal shape. The consistency of the rot is much the same as that of the normal fruit—hard when the fruit is green and somewhat soft when it is mature. In the field the surface of the rot on green fruits is smooth and without

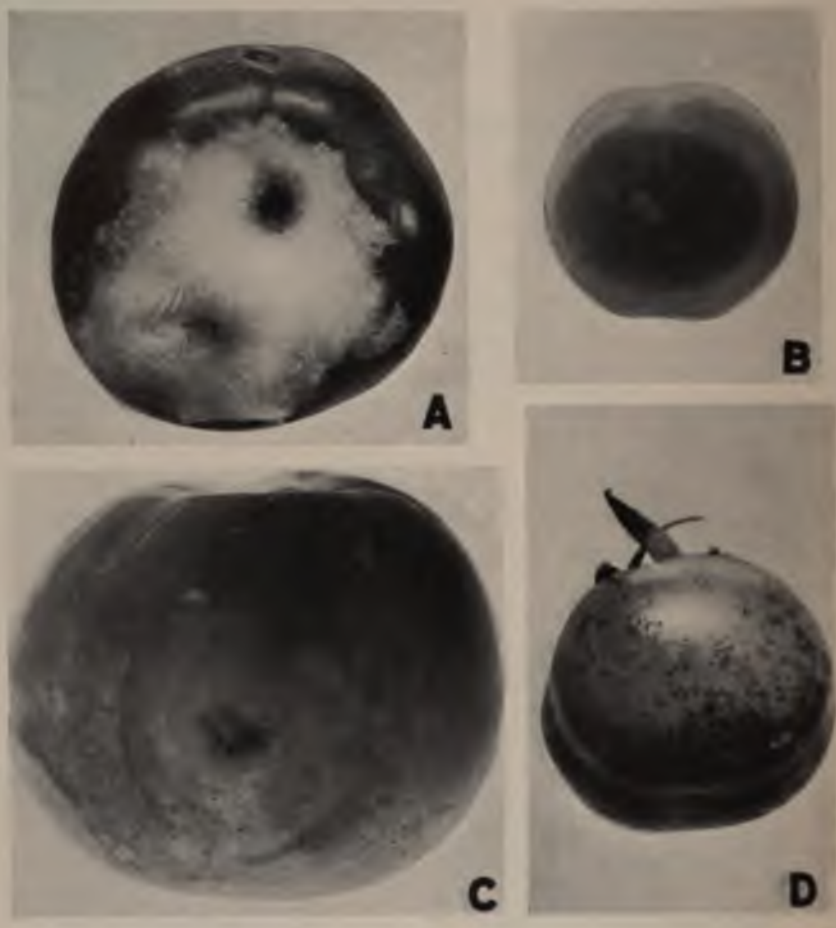

FIG. 2. TOMATO FRUITS INOCULATED WITH PHYTOPHTHORA TERRESTRIA

A, the fruit was kept in a moist chamber for ten days before the photograph was taken; under those conditions an abundant aerial mycelium is produced by the fungus. Natural size. B, green fruit inoculated from pure culture and kept one day in a moist chamber and then four days in open air before the photograph was taken; no aerial mycelium is produced under these conditions. × .75. *C*, a ripe fruit seven days after inoculation showing the rot produced by inoculation with swarm-spores; the dark spot is at the centre of the lesion; the affected area is 3.5 centimeters in diameter but is so near the color of the normal fruit that it does not appear distinctly in the photograph. Natural size. *D*, a green fruit, three days after inoculation with swarm-spores; the numerous infections appear in the form of minute, dark, scabby specks on the surface of the fruit. × .75.

any fungal growth. When the affected fruit is kept in an enclosure with high humidity, especially when the fruit approaches maturity, the fungus which causes the rot may be observed commonly on its surface (fig. 2*A*).

The rot occurs on the fruit in all stages of its development, beginning almost invariably at the point where the fruit touches the ground. Naturally, the fruit touches the ground most commonly with the blossom-end, on which account this rot often appears as a peculiar form of blossom-end rot and for which it sometimes has been mistaken.

NAME

The disease is known among some of the growers on the East Coast as water logged fruit. This name cannot be adopted for the rot because it is misleading and because it has no other advantage in its use. The name brown rot though it would often describe the disease, ought not be used in reference to the disease, first, because there are even more frequent cases when the color of the rot is not distinctly brown, and second, because the name has been applied already by Bancroft to an apparently different rot of tomato fruit.

Some of the tomato buyers use the name buckeye, in reference to a rot of tomato fruit. The writer was unsuccessful in finding with certainty what particular rot is called by that name, but his indirect information and observations indicate that the name refers to the rot under consideration. This name describes very well the most striking feature of the lesion on fruit affected with the disease, namely, its broad zonation, in which case the lesion indeed much suggests the eye of a large animal. The name also has not been used in literature before in reference to any disease of a similar nature. Therefore, it is suggested that the common name, buckeye, should be used in reference to the tomato fruit rot described here.

OCCURRENCE

The rot was found by the writer for the first time in January, 1915, at Goulds, Florida. Soon after the first observation and during the following three months it was found in every tomato field of that locality on the prairies—low marl lands usually under water during rainy summer months. The whole district is known as Redlands and lies at the extreme south end of the Florida East Coast, namely, south of Miami.

The same rot was observed by the writer in 1916 on the West Coast also. In April of that year it was found in a field near Bradentown, and in May in a shipment of tomato fruit received by the writer from a field near Palmetto. In both instances the tomatoes were grown on the common low hammock land of that vicinity.

The writer also found among some old specimens of tomato fruit preserved in formalin and kept in the laboratory at Gainesville, two specimens which by external symptoms and microscopic examination proved to be affected with this same rot. One of the specimens had a label indicating that it came from Little River, near Miami, in 1911. The other specimen bore no label.

All these observations show that the rot occurs in South Florida on both coasts where it is common in low fields even during a comparatively dry season, such as that of 1916. No actual observations were made of its occurrence in other parts of the state, but judging from its common presence in so widely separated parts as the east and west coasts and on soils so different in character, one might safely assume that it is much more generally distributed than observations indicate.

HISTORY

The preserved specimen of the rot found in the laboratory and previously mentioned, shows that the disease was present in the state at least as early as 1911. An inquiry among tomato growers of the East Coast also indicates that it has existed there a long time.

Pathological literature, with one exception, contains no reference to any disease of tomato fruit similar to the rot under consideration here. The organism which causes this rot is closely related to *Phytophthora infestans* but the latter fungus is not the same as the one which causes the rot herein described and the disease produced by *P. infestans* is also different because it affects all aerial parts of the plant and the fungus always produces abundant conidia on the surface of the affected parts of the fruit.

The previously mentioned exception is a short note by Bancroft[1] on The Brown Rot of the Tomato, in which is described briefly the symptoms, occurrence, economic importance, and the method of transmission of the disease. No illustrations of any kind accompany the article to assist in identification of the disease and in general it is too brief to judge with certainty whether the tomato fruit rot reported from England is the same as, or different from the rot found in Florida. It appears that the rot of tomato fruit reported by Bancroft has these two features similar to the buckeye rot: (1) The disease occurs only on the fruit; (2) the fungus associated with the rot in England is closely related to the fungus which causes the rot in Florida.[2]

[1] Bancroft, C. K. The brown rot of tomato. Jour. Bd. Agr. (London) **16**: 1012. 1910

[2] Whether it is really the same fungus or not it is impossible to determine, because about his fungus, Bancroft merely says "the tissues of the endosperm and embryo" of the affected seed "contain fungal hyphae, which from their characters

The points in which the two rots appear to differ are as follows: (1) In describing the rot in England, Bancroft says: "A fruit which is infected first shows discolored patches on its surface; these usually run together, so that the whole or almost the whole surface of the fruit becomes discolored." The rot observed in Florida as a rule appears in form of one, rarely more, gradually spreading, often distinctly zonate spot, usually at the blossom end of the fruit; (2) Bancroft from all his observations on the rot says this in regard to the way in which the disease affects the fruit: "This infected seed . . . . is known among the growers to be capable of germinating and is reported to produce plants which always bear infected fruits. These facts, coupled with the results of microscopic examination of the seed, suggest that the hyphae may be capable of existing in the seed in a resting condition, becoming active when the seed germinates, and keeping pace with the growth of the plant until the fruit is formed." That is, Bancroft evidently records no other method of infection of the fruit than the one suggested in the above citation, while it is evident that in the case of the rot in Florida the infection starts from outside the fruit. From the above citation it is also evident that the rot reported by Bancroft is associated rather with the use of infected seed (the plants from infected seed "always bear infected fruits") while in our case it evidently is associated with the infested soil, and only the fruits which touch or nearly touch the ground are affected with the rot.

On the whole, it seems that the disease described by Bancroft is different from the rot of tomato fruit found in Florida, and the latter should therefore be considered as a new one to the literature.

ECONOMIC IMPORTANCE

The data at hand will not justify any precise statement concerning the extent of damage caused by the rot. But considering that the tomato crop is the most important of all vegetable crops in Florida, and considering also that the rot was actually observed to affect up to fifteen per cent of the fruit in the field and up to ten per cent of the fruit in transit, it is evident that the disease is important.

CAUSE

Repeated isolations from the tissues of tomato fruit affected with the rot invariably yielded one and the same fungus, usually in pure cultures di-

appear to be hyphae of *Phytophthora omnivora*." Hyphae of a number of fungi belonging to the same family do appear under similar conditions alike, the fungus causing the rot in Florida included; and yet they are quite different, but to differentiate them much more than appearance of the hyphae is needed.

rectly from plantings. The first isolations were made from the material collected on the East Coast in January, 1915; the last in May, 1916, from material obtained from the West Coast.

The isolations were made by planting small bits of the affected tissues (from under the epidermis and after the fruit was disinfected in mercuric chloride solution, 1 : 1000, for about fifteen minutes) into either petri dishes with a suitable medium (corn-meal and oat agars were most commonly used for this purpose) or into test-tubes with sterilized bean pods or oat agar. Dilutions of swarm-spores were employed to make certain that the cultures were pure. This was an easy procedure because the fungus readily sporulates and the swarm-spores are produced from mature conidia (swarmsporangia) in a very short time and practically under all conditions of the laboratory (under favorable conditions swarm-spores were produced in some instances eight minutes after the culture was placed in fresh water). A very successful procedure here is to place a good-sized piece of bean-pod culture (two or more weeks old) of the fungus into a sterilized watch-glass, with a few cubic centimeters of sterilized water in it, for about forty-eight hours; then, after the culture in the watch-glass is washed a few times in fresh sterilized water, it is left in the watch-glass with a few cubic centimeters of water for half an hour. In the first two days the bean-pod culture usually produces a great mass of conidia; in half an hour or an hour after it is rinsed, these swarmsporangia will liberate into the water a considerable number of swarm-spores; this water with numerous swarm-spores in it is to be used then for poured-plate dilutions made in the ordinary way. Corn-meal agar was commonly and successfully used by the writer for these dilutions.

Inoculations of tomato fruit, detached from the plant, and not detached, and of all stages from very young to red-ripe with pure cultures of the fungus, invariably resulted in reproduction of the rot (fig. 2). The inoculations were made by placing small bits of the culture and water suspensions of swarm-spores upon wounded and unwounded surfaces of the fruit. In the case of swarm-spore inoculation a piece of the fungus culture was dropped into a large jar nearly full of sterilized water and then the fruit was placed into the water. Detached tomato fruits float in water and undetached fruit can be conveniently placed in such a way that only a part of it will be in the water.

Inoculations into wounded fruit nearly always showed the rot in twenty-four hours. Inoculations into unwounded fruit, whether mycelium or swarm-spores were employed, sometimes showed the rot in twenty-four hours after the inoculation was made, but often the infection could not be detected until three or even four days later. At present no explanation is offered for this variation in the incubation period.

Repeated reisolations were made from artificially infected fruit. These uniformly yielded again the same fungus, leaving no doubt that this fungus is the cause of the buckeye rot here described.

THE FUNGUS

The fungus which causes the buckeye rot of tomato fruit has all the characters—the formation of swarm-spores within the conidium and their escape individually or in groups included—of the genus Phytophthora and therefore should be classed with members of that genus in spite of its soil habitat (fig. 5).

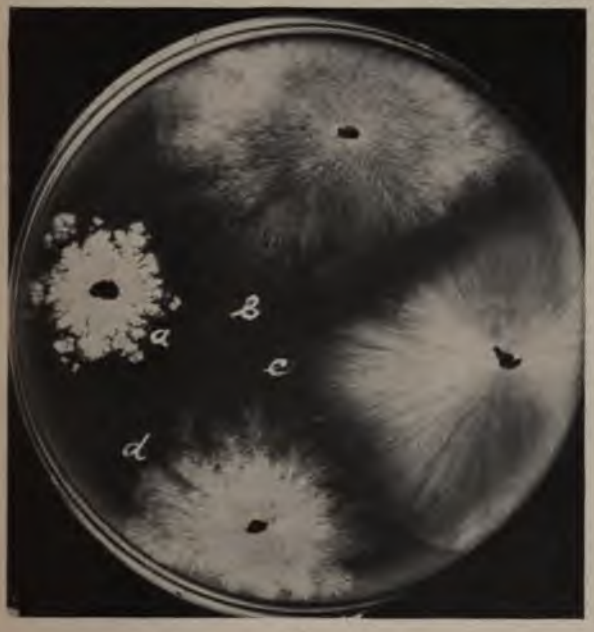

FIG. 3

FIG. 4

FIG. 3. CULTURES OF P. TERRESTRIA AND RELATED SPECIES

The medium is corn-meal agar, age twelve days, natural size. The characteristic tufted growth of P. terrestria on this medium is markedly different from that of related species. *a*, Phytophthora terrestria; *b*, Phytophthora erythroseptica; *c*, Phthiacystis citrophthora; *d*, Phytophthora cactorum.

FIG. 4. SEXUAL ORGANS OF PHYTOPHTHORA TERRESTRIA

A fertilized oogonium with antheridium at the base. The stalk of the oogonium evidently penetrates the antheridium. From four-months-old culture on steamed bean pod. × 1240.

The fungus has been grown and studied on various media parallel with other organisms related to it, such as *Pythium debaryanum* Hesse., *Pythiacystis citrophthora* Sm. & Sm., and several species of Phytophthora, *P. cactorum* (Lebert & Cohn) Schr. and *P. erythroseptica* Pethybr. included. This comparative study shows plainly that the fungus causing the buckeye rot of tomato fruit is different from the others. Its peculiar tufted growth on the corn-meal agar[3] in plates, is one of the differentiating characters, especially valuable for an easy separation of this fungus from the others (fig. 3).

Examination of the literature also indicates that this fungus has no been previously described. However, evidently the same organism wa isolated before, though from another host; but it has been considered to be the same as the fungus of the lemon brown rot, namely, *Pythiacystis citrophthora* Sm. & Sm. The reference here is made to the organism isolated by H. S. Fawcett[4] and by H. E. Stevens from the bark of citrus trees in Florida affected with the foot rot.

The writer's comparative study of the fungus of the buckeye rot of tomato, of the fungus of the lemon brown rot, *P. citrophthora*,and of the fungus from the citrous foot rot in Florida shows that the tomato rot organism and the one of the citrous foot rot are morphologically and culturally identical and that it is distinctly different from the true *P. citrophthora*.

A fungus was recently isolated by the writer from a lupine stem rot which is evidently the same as the one of the buckeye rot of tomato fruit.

Inoculations of tomato, sweet pepper, watermelon fruit, of lemons and of tubers of Irish potatoes with pure cultures of the several species of Phytophthora, previously mentioned, of the Pythium, Pythiacystis and three strains of the fungus of the buckeye rot, including one strain isolated by H. E. Stevens from diseased bark of a citrus tree in Florida affected with the foot rot, show that certain organisms distinctly different in their morphology may affect the same host and produce more or less similar effects. The fungus of the buckeye rot produced a rot of all the parts of the plants here referred to.

These inoculations thus indicate that the organisms which do attack the same plant or plants cannot on this basis alone be considered identical with each other.

[3] The corn-meal agar is made by heating 50 grams of corn-meal in 1000 cc. of distilled water at 60 C. for an hour, then filtering the liquid through a filter paper, adding to it 15 grams of agar and the required amount of distilled water to bring volume of the liquid to 1000 cc., cooking the substance in a double boiler until all agar is dissolved, cooling down to 50 C., adding the white of an egg, autoclaving at 15 pounds of pressure for about fifteen minutes, filtering through a filter paper, tubing and autoclaving as before

[4] Fawcett, H. S. The known distribution of Pythiacystis citrophthora and its probable relation to mal di gomma of citrus. Phytopath. 5: 66, 67. 1915.

On the basis of the data obtained in the course of the writer's comparative study of the fungus of the rot of tomato fruit and of the other fungi related to it, it is considered an undescribed species which may be briefly characterized as follows:

Phytophthora terrestria n. sp.

Mycelium at first continuous then septate; conidia usually terminal, sometimes intercalary, mostly oval, papillate at apex but variable, 42.5 × 30.5 (36–46 × 24–35)μ, germinating mostly by swarm-spores; swarm-spores asymmetric, with two cilia on one side, 9.5 (9–11)μ, when in resting, globoid stage; chlamydospores common, mostly globose, 34 (30–40)μ; oogonia common in old cultures on steamed bean pods, globose, 22 (19–24)μ with the stalk evidently penetrating through large, nearly globose antheridium (fig. 4); oospores globose, 20 (18–21)μ; colonies on corn-meal agar, in petri dish, peculiarly tufted.

Hab. Parasitic in tomato fruit causing buckeye rot, in bark of trunks of citrus trees causing foot rot, in stems of a Lupine causing stem rot, and apparently in low soils; in Florida.

Phytophthora terrestria sp. nov.

Mycelio priuo continuo deinde septato; conidiis fere terminalibus aliquando intercalaribus, plerumque ovoideis apice papillatis sed valde variabilibus, 42.5 × 30.5 (36–46 × 24–35)μ, per zoosporos fere germinatis; zoosporis asymmetris, lateraliter 2-ciliatis, 9.5 (9–11)μ diam. quum quieti, globosi statu; chlamydosporis vulgaribus, plerumque globosibus, 34 (30–40)μ diam.; oogoniis globosis, 22 (19–24)μ diam., radicibus per amplum sub-globosum antheridium aperte penetrantibus; oosporis globosibus 20 (18–21)μ diam.; coloniis in agaro Zeae Maydis farina, in petri patera, suo genere cristati.

Hab. parasitice in fructibus Lycopercici esculenti efficiens "buckeye rot," in trucis Citri efficiens "foot rot," in caulibus Lupini sp. efficiens "stem rot," et aperte in humilibus solis, Floridensibus, Am. bor.

CONTROL

No direct control experiments with the buckeye rot have been conducted, but the fact that the rot occurs almost invariably only when the fruit touches the ground, or is very close to it, naturally suggests staking the tomato plants to prevent it. The staking would hold the fruit high enough above the ground to keep it from attack by the fungus.

The fact that the fungus attacks the fruit from the ground, coupled with the fact that the rot, once it starts, progresses under normal conditions fairly rapidly (often nearly the entire fruit may become affected in

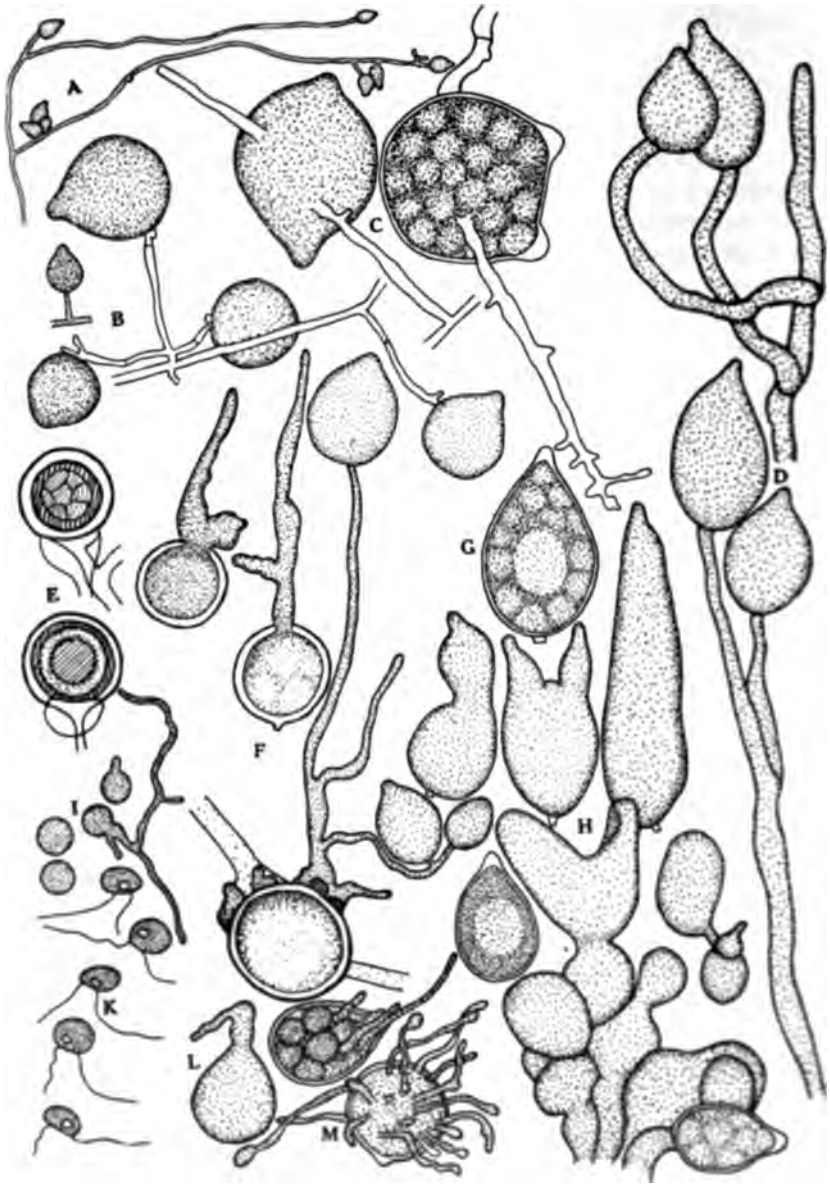

FIG. 5. PHYTOPHTHORA TERRESTRIA

three days after inoculation, the rate depending evidently on the temperature, moisture content, and maturity of the fruit), also suggests a method of control of the rot while in transit. Here it probably would be very advantageous to keep the fruit for a few days after it is picked before packing it for shipment. All fruits that were infected in the field would develop the rot sufficiently to be detected by the packers and thus be thrown out without contaminating the rest of the fruit, which then could be safely packed and shipped. But the practicability of these methods has not been tested.

SUMMARY

1. The buckeye rot of tomato fruit is common in certain places on the low lands of the east and west coasts of Florida.

2. It occurs only on the fruit that touches or nearly touches the ground.

3. It is caused by the fungus *Phytophthora terrestria* n. sp., which is also found on other hosts.

4 It causes considerable injury to the fruit in the field and in transit.

5. Staking of the plants in the field and keeping over the picked fruit a few days before it is packed seem to be practical methods of its control.

UNIVERSITY OF FLORIDA AGRICULTURAL EXPERIMENT STATION
GAINESVILLE, FLORIDA

EXPLANATION OF FIG. 5

A, conidiophores from culture on hard, oat agar, eight days old. × 100. *B*, conidiophores and *C*, intercalary conidia; from the same medium and of the same age as *A*. *D*, conidiophores from the fungous growth on an artificially inoculated (upper) and on naturally (lower) affected and nearly mature tomato fruit. *E*, oospores within the oogonia with the basal antheridia evidently penetrated by oogonial stalk. *F*, germinated chlamydospores. *G*, typical and *H*, various abnormal forms of conidia, produced on surface of a mature tomato fruit affected with the buckeye rot. *I*, resting and germinating swarm-spores. *K*, motile swarm-spores (fixed in Flemming's fluid and stained with eosin). *L*, conidium germinating by a single germ tube. *M*, two conidia germinating by many germ tubes (evidently each swarm-spore which did not escape gives its own germ tube). *B–L* × 500.

NOTEWORTHY PORTO RICAN PLANT DISEASES

F. L. Stevens

The following diseases are of interest to pathologists of the United States, either because they are new, or because they are little known diseases of important crops belonging within the territory of the United States or are caused by fungi of special interest for one reason or another.

Coffee. *Pellicularia koleroga.* This fungus occurs in great abundance, especially in the lower altitudes in Porto Rico. In destructiveness and general appearance it gives an impression somewhat like that of pear blight with many branches killed and the dead leaves matted and hanging by their fungous attachments. The fungus forms thin skin-like membranes over the lower leaf surfaces and has thread-like growths leading down the petiole and adjacent branches. In habit it strongly resembles *Hypochnus ochroleucus* Noack as it occurs on apples in the Carolina mountains, and the writer long has had the suspicion that the two fungi are related.

Hemileia vastatrix. This destructive fungus, though not seen in Porto Rico, is worthy of mention on that account. There is a report that it was imported into the island, almost immediately recognized, and due to the vigilance of the experiment station officials was so thoroughly eradicated that not a specimen has since been collected. The following quotation from a letter from Mr. May, in charge of the Porto Rican Experiment Station, is worthy of record.

"When the station was first established in Porto Rico we were carrying on some experiments in the Carmelita coffee plantation, five hours by horseback above Ponce, under charge of J. W. Van Leenhoff. In 1902 or 1903, I do not remember which, it was before I came to the station, Van Leenhoff got some little coffee trees from a Dutch warship that brought them from Java. After he planted them out he noticed that they had what appeared to be *Hemileia vastatrix.* Mr. Van Leenhoff had been a coffee planter in Java and was pretty sure of the fungus from such investigation as he could make. The matter was communicated to Washington and L. A. Clinton, of Connecticut, was sent down to investigate the trouble. In the meantime Van Leenhoff took every precaution, destroying all the plants and all material that might have in any way been connected with them. Clinton spent some weeks at the Car-

melita but could find no traces of the fungus and as none has appeared since, Van Leenhoff doubtless made a 'clean up.' Since that time great care has been exercised with all coffees brought from foreign countries."

Stilbella flavida. The characteristic circular leaf spot caused by this fungus is common in the higher altitudes, but is never found in low regions. The fungus is of unusual scientific interest on account of its problematic relationships. It is by no means limited to coffee but is found on numerous hosts.

SUGAR CANE. *Leptosphæria Sacchari.* This fungus is quite commonly present as a destructive leaf spot.

PALM. *Graphiola Phoenicis.* This fungus, which is of uncertain relationship and is common as a minor pest in northern greenhouses, is found in the open on several species of palms in Porto Rico. It has been noted particularly upon the date palm and the hat palm. The hat palm is a very profitable plant, a single leaf being worth about ten cents. Frequently trees are seen with all the leaves closely covered with Graphiola and lines of diseased tissue reaching long distances through the petioles. The financial damage is done to the young, as yet unfolded leaves, the fungus penetrating them and rendering them worthless.

Meliola furcata. This is present sometimes to such extent as to largely cover the leaves with its black coating. It has not been observed on cultivated palms but occurs in great abundance on wild *Thrinax ponceana* on plants of marketable size. As yet, however, there seems to have been no attempt to make a commercial enterprise of shipping the beautiful young palms which spring up spontaneously in such profusion in Porto Rico.

Auerswaldia palmicola. This was noted only a few times, but in those instances affected nearly every leaf and leaf-segment on the tree.

BREAD FRUIT. *Uredo Artocarpi.* The immense leaves of the bread-fruit harbor numerous fungi. The crop, if it can be called such, is not of high value, and this rust, though interesting, is not of much economic significance.

CORN. *Phyllachora graminis.* Mention has been made of this in PHYTOPATHOLOGY by Miss Nora Dalby. The disease was wide-spread in Porto Rico and must have been to considerable extent injurious.

BEAN. *Dimerium grammodes.* This striking fungus which occupies and covers the veins of the affected parts of the leaf with its conspicuous black perithecia is common on several genera of legumes, among them cultivated beans.

GUAVA. *Meliola Psidii.* The black spots of this fungus are almost universally present wherever the host is found. There is but little, if any injury.

Aschersonia. What is taken to be one of this genus sometimes occurs profusely on the lower surfaces of guava leaves, giving them a livid scarlet color, conspicuous to a considerable distance. The fungus appears to be growing upon a scale insect, probably *Alerodes citri.*

Cephaleurus virescens. This algal parasite is of special interest. The spots show well from both below and above the leaf, the leaf tissue being killed. This alga, also widely known as the cause of a serious tea disease, is present parasitically on a large number of Porto Rican plants.

GONDULE. *Uromyces dolicholi.* This universally present leguminous plant is almost always rusted to a slight degree, sometimes badly rusted.

MANGO. *Meliola Mangiferæ.* Meliola is very common and widespread but apparently not injurious. *Gloeosporium mangiferæ* is often abundant and injurious, especially upon the fruits of the finer varieties of the mango. The effect in general is much like that of the bitter rot on apple.

SWEET POTATO. *Coleosporium Ipomœæ.* Rust is common on this and other Ipomœas, but usually not to serious extent. In one field at Preston's Ranche near Naguaba, infection was general and serious. Each sorus was also parasitized by a Fusarium-like fungus, rendering it white.

GRAPE. *Physopella vitis.* This is a rust of cultivated grape. Three collections were made in Porto Rico but all from the same vines, namely, at Patillo Springs. The rust was present in quantity sufficient to make it injurious. The writer knows of only a few grape vines in Porto Rico. If there were more vines perhaps there would have been more collections of this rust.

PEANUT. *Uromyces arachidis.* Only one collection of this rust was made. Indeed, the peanut plant is not very common in Porto Rico.

CANNA. *Puccinia Cannæ.* The rust on Canna is common on both wide and cultivated Cannas in all parts of Porto Rico; on nearly every one of the host plants. Sometimes the rust is so abundant as to appear fairly destructive.

INGA LAURINA. *Microstroma* sp. This host, an important leguminous coffee shade tree, is frequently much infested with an undescribed Microstroma which causes large witches brooms.

PITHECOLOBIUM SAMAN. *Microstroma* sp. This leguminous tree is being introduced into Porto Rico through the efforts of the experiment station, and in the seed beds and propagating beds it is frequently heavily infested with an apparently undescribed species of Microstroma.

PASPALUM. *Myriogenospora* sp. The Paspalums of yard and pasture often bear this very interesting fungus. Infection is usually general throughout the plant, that is, if one part is infected each leaf is likely to be invaded.

MELIA. *Pseudoperonospora* sp. Two collections were made, in widely separated parts of the island, of this fungus. The damage is probably slight. The four fungi last mentioned will be described fully by Dr. Lamkey.

FICUS. *Kuehneola Fici.* This rust was very conspicuous and present on a large number of species of this genus. Certain large trees at the proper period of the year were repeatedly covered with the rust and often small shoots a foot or so high would have each leaf completely rusted.

COTTON. *Kuehneola Gossypii.* Very little cotton is raised in Porto Rico. This rust was found in considerable abundance in one field, though probably not doing much damage.

MANIHOT. *Uromyces Janiphæ.* Only one collection of this rust was made in Porto Rico, though the host is very common there under cultivation, and the rust has been looked for repeatedly.

SOOTY MOLDS. The sooty molds familiar to Northern pathologists on the orange and Camellia and to lesser degree on many greenhouse plants, abound in Porto Rico. There seems to be no specialization to hosts and they grow indiscriminately upon all hosts. If it be a large plant as a mango tree that is primarily infected, the fungus, spread doubtless by rain, is found growing upon every kind of plant beneath the tree. Very little evil effect is noticeable other than the unsightly condition produced. The statement is frequently seen that these sooty molds exist upon insects or insect secretions. Such organic matter certainly favors them and increases their luxuriance, but the sooty molds are not entirely dependent upon insects and insect products and may abound without them.

While many of the Porto Rican sooty molds are much like, perhaps quite like, the sooty mold of the orange, other sooty molds diverge more or less from this in character, yet show similarity enough among themselves to allow them to be classed in the same group.

The writer has so far refrained from using latin names for these fungi. The orange and camellia sooty molds are best known under the generic name Meliola, and the generic conception of Meliola was broad enough to receive them until about 1892. Gaillard's monograph of Meliola then showed that these forms clearly differ greatly from the typical Meliolas in essential details, and he excluded from Meliola the tropical sooty molds. Today any student of Meliola would, I think, agree with him. Meliola with its capitate and mucronate hyphopodia, its characteristic mycelium, perithecia, asci and spores constitutes a well-defined genus from which the sooty molds with their bead-like mycelium, peculiarly shaped pycnidia and perithecia differ widely. The fact that they should

be excluded from Meliola is clear. Just where they should be placed is, however, not so clear. They comprise a rather well-defined group which in Porto Rico certainly consists of a large number of species on many hosts. This group perhaps coincides in limits with what Saccardo in his earlier volumes calls the sub-family Capnodieæ and which he in his fourteenth volume calls a tribe. This tribe is not recognised by Lindau in the Naturlichen Pflanzenfamilien, but is included in the Perisporiaceæ. Clements gives the Capnodiaceæ family rank. Arnaud in 1911 in his monograph "Contribution a l'etude des Fumagines" Ann. Ec. Nat. Agr. Montpellier, places them for convenience in the "Spheriacees dictyosporees." Many writers today place the better-known of these forms of the habit of the old *Meliola Citri*, *Meliola Camellæ*, and so forth, in the genus Capnodium. Thus we have *C. stellatum*, *C. Mangiferum*, *C. Coffeæ*, *C. brasiliense*, *C. footie*. Others place these forms in the genera Pleosphæria, Antennaria, Apiosporium, and so on. It is not the present purpose of the writer to attempt to determine the generic limits here or the status of these species, but rather to call attention to the rich mass of material occurring in the tropics, which may well be called the Sooty Molds and in the main belong to the Capnodieæ of Saccardo.

ILLINOIS UNIVERSITY
URBANA, ILLINOIS

PYCNIAL STAGES OF IMPORTANT FOREST TREE RUSTS

JAMES R. WEIR AND ERNEST E. HUBERT

WITH TWO FIGURES IN THE TEXT

The discovery on September 29, 1916, at Darby, and on October 8, 1916, at Bonner, Montana, of abundant exudations of pycnospores on swellings of *Pinus ponderosa* (fig. 1) and *Pinus contorta* caused by *Cronartium Comandræ* Pk. somewhat alters the impression that the pycnia of the caulicolous species of forest tree rusts appear only during the spring or early summer months. This unusual appearance of pycnospores seems to be of sufficient importance in the life history of this rust to be reported at this time together with further facts concerning the pycnial stages of *Cronartium coleosporoides* (D. & H.) Arthur,[1] *Cronartium Comptoniæ*,[1] Arthur *and Cronartium cerebrum* (Pk.) H. & L. Spaulding[2] in his accounts of the white pine blister rust (*Cronartium ribicola* Fisher) states that the pycnial stage may be found early in the spring or at almost any season in late summer or fall, he having found them on the hosts in November within a month after placing in the greenhouse. The collections of *Cronartium Comandræ* on *Pinus ponderosa* and *P. contorta* bearing the pycnial stage were of the spindle-shaped type of swellings and bore in the central portion cankerous corrugations of the current season's (May, 1916) aecial eruptions. The pycnial drops appeared on the freshly swollen areas at either end of the spindle-shaped hypertrophies bordering the ruptured areas. This conforms to observations made on a collection of the pycnial drops made near Bonner, Montana, on May 22, 1916, from lesions of *Cronartium Comandræ* on *Pinus ponderosa*. The pycnial exudations, consisting of a clear, sticky, sweet liquid with a large number of minutely pyriform spores in suspension, appear as large or small drops issuing from minute blister-like swellings in the epidermis of the infected tissues. Measurements of the pycnospores of the various collections made agree closely with those made by Boyce,[3] (50) 3 to 4 μ by

[1] Weir, J. R. and Hubert, E. E. Recent cultures of forest tree rusts. Phytopath. 7: 106–109. 1917.

[2] Spaulding, Perley. The blister rust of white pine. U. S. Dept. Agr. Bur. Plant Ind. Bul. 206: 27–28. 1911.

———. The white pine blister rust. U. S. Dept. Agr. Farmer's Bul. 742: 12. 1906.

[3] Boyce, J. S. Pycnia of Cronartium pyriforme. Phytopath. 6: 446–447. D. 1916.

3 to 7 μ (3 by 4). They are characteristically pyriform, of a pale turtle green color[4] issuing from minute openings in the epidermis and are produced from subepidermal pycnial stromata of irregular outline. Boyce states that the pycnospores are hyaline. An examination of both isolated and massed spores indicates that they are colored, though faintly.

The pycnial stages of *Cronartium coleosporoides* and *Cronartium Comptoniæ* have, up to the present, remained unknown. Mention of the discovery of the stage has been made in a previous report[5] but details were not given at that time. In the period from April 4 to 15, 1916, abundant pycnial exudations containing pycnospores were obtained from galls of *Cronartium coleosporoides* (fig. 2) on *Pinus ponderosa* and *P. contorta*. These pycnial drops were found in the field and were also forced in the laboratory at a much earlier date than produced in nature.[6] Out of a total of 32 galls, 28 produced pycnia by the forcing process, several of these later producing aecia. The pycnia of *Cronartium coleosporoides* on galls develop similarly to those of *Cronartium Comandræ* with one marked difference, that is, their appearance on old galls and lesions. The pycnospores of *Cronartium Comandræ* apparently develop but once on the same tissue preceding the appearance of the aeciospores. The production of aecia kills the infected tissues which are included in the aecial ruptures. The tissues bordering this area are invaded by the mycelium of the fungus, produce swellings, and give rise to pycnospores, either in early spring or in late summer and fall, whenever sufficient time has elapsed from the last production of aecia. In the cases recorded the pycnospores appeared in the same season following the production of aecia, with only five months intervening, but not from the identical area from which the pycnia were produced. In *Cronartium coleosporoides* the pycnospores are produced on old galls previously ruptured as well as on unruptured infected tissues. A description of the pycnia of *Cronartium coleosporoides* on galls follows:

Pycnial stroma in irregularly shaped areas, more or less scattered or anastomosing, caulicolous, subepidermal, forming minute, blister-like swellings when mature on unruptured infected tissues and issuing from cracks in the bark of old lesions; exuding a clear, sweet, sticky fluid in which the pycnospores are suspended forming drops of a cadmium yellow to orange color when first appearing, becoming clear as the spore mass settles to the lower end of drop, and orange to brick-red upon drying. Pycnospores hyaline, mostly spherical, occasionally ellipsoid or obovate. (50) 1.5 to 3.0 μ by 1.5 to 3.7 μ (2.5 by 2.5).

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[4] Ridgeway, Robert. Color standards and color nomenclature. Pl. 32. 1912.
[5] Phytopath. 7: 106 109. 1917.
[6] The same.

Several specimens of *Cronartium Comptoniæ* bearing pycnial drops were secured by laboratory forcing methods following collection in the field. The material was collected on *Pinus banksiana* at Cass Lake, Minnesota, on May 12, 1916, placed in large test-tubes with water May 15, and developed pycnia on May 17 and 18. The pycnospores develop similarly to those of *Cronartium Comandræ* in respect to their appearance on pre-

FIG. 1. PYCNIAL EXUDATIONS OF CRONARTIUM COMANDRÆ ON LESION OF PINUS PONDEROSA

Note the large number of drops on the lesion

viously unruptured tissues. Here the aecial production also tends to kill the immediate tissue, in many cases girdling the stem. The following is a description of the pycnial stage of *Cronartium Comptoniæ*:

Pycnial stroma in irregularly shaped areas, more or less scattered or anastomosing, caulicolous, subepidermal, forming minute blister-like swellings when mature on unruptured infected tissues; exuding a clear, sweet, sticky fluid in which pycnospores are suspended forming drops of a cadmium yellow color when first

appearing, becoming clear as the spore mass settles to lower end of drops, and brazil-red upon drying. Pycnospores hyaline, ovoid to ellipsoid, very rarely pyriform. (50) 1.6 to 4.3 μ by 1.6 to 6.0 μ (2.2 by 4.0).

On May 12, 1916, a large collection of galls of *Cronartium cerebrum* (Pk.) H. & L. on *Pinus banksiana* were collected at Cass Lake, Minnesota. These were placed in large test-tubes with water in the laboratory

FIG. 2. PYCNIAL EXUDATIONS OF CRONARTIUM COLEOSPOROIDES ON GALLS OF PINUS CONTORTA

Note the settled spore masses in lower portion of drops

on May 15. Some of the galls bore pycnia when collected but the majority of pycnia appeared after forcing in the laboratory and continued producing pycnial drops up to May 17. On May 17, four of the galls developed aecia within four to five millimeter distance from the pycnial exudations on the same galls but not from identical pycnial areas. Out of twenty-eight galls, twenty-three produced pycnia in abundance and of these, eighteen also produced aecia. Several of the galls when received

from the field bore remnants of the pycnial exudations and at the same time were producing aecia in abundance. The pycnial drops when fresh have a slightly darker yel'ow color than those of *Cronartium coleosporoides*, and like the latter appear in the bark crevices of old galls as well as in minute blister-like swellings on the younger unruptured tissues of infected areas. When old and dried the pycnial exudations are difficult of detection on the surface of the infected tissues and have a brick-red color.

From observations made on *Cronartium coleosporoides* on galls both in the laboratory forcing experiments and in the field, it is determined that the aecia follow the pycnia in the same season, usual'y from eight to sixteen days apart but not appearing upon the identical areas. The galls shown in figure 1 were collected at Coeur d'Alene April 1, 1916, and were placed in test-tubes with water on April 3. From April 4 to 9 abundant pycnial drops were produced. From April 11 to 14 the galls began to show evidence of aecial eruptions and from April 16 to 17 aecia appeared. With a longer time-interval between the two stages this is what has been observed to occur in the field. Spaulding[7] has observed in the case of *Cronartium ribicola* that the pycnia precede the aecia by a short period.

A very interesting fungus frequently found during the past season in connection with the pycnial exudations and also found accompanying the aecial stage of the caülicolous rust is a species of Tuberculina which may be referred to *T. maxima* Rostrup.[8] This fungus was found attacking the pycnial and aecial stages of *Cronartium Comandræ* occurring on *Pinus ponderosa* and *P. contorta*, the pycnial and aecial stages of *Cronartium coleosporoides* on galls of *Pinus ponderosa* and *P. contorta*, and the pycnial and aecial stages of *Cronartium cerebrum* on *Pinus banksiana*. This species of Tuberculina attacks the stromatal layer as well as the fruiting bodies and erupts in powdery, lilac to nigrosin masses through the epidermis of the host tree. This fungus is considered by Tubeuf[9] to be of some economic importance although Lechmere[10] does not concur in this conclusion. The writers have not found it occurring in sufficient abundance to place any importance upon its economic possibilities.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
MISSOULA, MONTANA

[7] U. S. Dept. Agr., Bur. Plant Ind. Bul. 206: 27–28. 1911.

[8] Tubeuf, C. von. Ueber Tuberculina maxima, einen Parasiten des Weymouthskiefern-Blasenrostes. Biol. Abt. f. Land- u. Forstwirthschaft. 2: 169. 1901.

[9] Tubeuf, C. von. Recent observations on the blister rust of Weymouth pine. Naturw. Ztschr. Forst.-u. Landw. 12: 484–491. 1914.

[10] Lechmere, E. Tuberculina maxima, a parasite on the blister rust fungus of the Weymouth pine. Naturw. Ztschr. Forst.- u. Landw. 12: 491–498. 1914.

REVIEWS

Edible and Poisonous Mushrooms. By W. A. Murrill, Assistant Director of the New York Botanical Garden. Handbook, 16mo., pp. 71, large colored chart, figs. 47. Published by the Garden. Price $2.

This work is primarily of interest to users of mushrooms. The chart contains very good illustrations of 30 species of edible mushrooms, and of 17 species of poisonous ones. A few of these are of interest to the forest pathologist, because they frequently attack the wood of living trees.

GEO. G. HEDGCOCK

PHYTOPATHOLOGICAL NOTES

New hosts for Razoumofskya americana and R. occidentalis abietina. *Razoumofskya americana* Nutt, has been previously reported on *Pinus contorta, P. banksiana, P. ponderosa,* and *P. jeffreyi.* On April 12, 1915, a specimen was received from J. E. Haefner of the Siskiyou National Forest, Oregon, on *Pinus attenuata.* On September 5, 1916, the writer collected both staminate and pistillate plants on *Pinus attenuata* in the Oregon Mountains, Siskiyou Forest. This indicates that the species may be expected to occur on any of the yellow pines. (See writer's note, Phytopath. **6**: 414. 1916.)

Razoumofskya occidentalis abietina (Engelm.) Coville, the large form on Abies, has been reported on *Abies concolor, A. grandis,* and *A. magnifica.*[1] During a trip on the Crater National Forest, Oregon, in September, 1916, the writer collected both staminate and pistillate plants on *Abies nobilis* and *A. amabilis.*

JAMES R. WEIR

Lightning injury to kale. In a previous article (Phytopath. **5**: 94. 1915) the writer, jointly with Gilbert, reported observations upon lightning killing of potatoes and cotton. As shown, the plants in the stricken zone die promptly over a somewhat circular area usually from one to three rods in diameter. Further evidence of this sort of injury in Wis-

[1] Hedgcock, G. G. Notes on some diseases of trees in our national forests. V. Phytopath. **5**: 176. 1915.

Weir, J. R. Mistletoe injury to conifers in the northwest. U. S. Dept. Agr. Bul. 360: 33. 1916.

consin potato fields has since come to us, also reports of like occurrence in sugar beet fields, although no example of the latter has come under our personal observation. It was of especial interest this summer to find a fully authenticated case of lightning injury to kale (fig. 1). This occurred on the trial grounds of D. M. Ferry & Company at Rochester, Michigan, about twenty-five miles northwest from Detroit. The lightning struck during the early part of a thunder storm, July 20,1916. The stricken spot was in the center of a bed of curly kale (*Brassica oleracea* var. *acephala*). Mr. Will Coulter, superintendent of the grounds, was

FIG. 1. LIGHTNING INJURY TO KALE

Note the white stake about the center of the dead area, marking the spot where the lightning struck.

walking a few rods away from this spot at the moment and was knocked to the ground, where he lay unconscious for a brief period. We visited the garden about September first, when Mr. Coulter kindly furnished us with the photograph of the field and the following data. Since he is a critical and trained observer, it may be accepted as accurate. "The white stake near the center of the bare area shows where the bolt actually struck. At this point there was made a nearly circular depression about 2.5 feet across and six to eight inches deep, and extending downward from the center of this depression as far as we could see was an irregular shaped hole about 2.5 inches in diameter. The kale plants at the time

of the storm were rather small and all had the appearance of having been struck by some strong force and flattened to the ground, and the three plants which seem to be inside the circle and have survived were almost completely covered with earth. The rest of the plants in the circle had a powder-burned appearance and all shrivelled up and disappeared within a week or ten days." The area as shown in the figure was roughly circular, about twenty to twenty-five feet in diameter. This accords closely in all respects with the effects of the lightning strokes as we have observed them in Wisconsin potato fields.

It is to be hoped that others may record, as they have opportunity, evidence of such injuries until we have a fuller understanding of these matters. From the evidence at hand, similar injuries may be expected with sugar and garden beets, and possibly carrots, with the various members of the cabbage-kale groups of vegetables, turnips, radishes, and so forth, and probably with the allies of the potato, such as tomato and egg plants. Special attention may also well be given to possible lightning injury of legumes in view of Sitensky's observations. (See Abstract, Zeitschr. Pflkr. **8**: 148, 1898), which have come to my attention since our former article. He reports a lighting stroke in a Bohemian alfalfa field when the plants were in blossom. The next day the plants were wilted down in a circular area about 5 meters across.

It is noteworthy that no case is recorded of like injury with any of the Gramineae, although it would seem that, since great areas are occupied by the grains and grasses in the northern states, lightning must often strike in such fields. When more evidence is at hand concerning the varying liability of such plants to injury in nature it will pave the way to some very interesting experimental work to determine the reasons why such variations occur. These may conceivably be associated with differences in the character or habit of aerial parts, with the character or distribution of the root systems, or with the relative electrical conductivity of the different plant tissues.

L. R. JONES

Puccinia glumarum. In May, 1915, the occurrence of *Puccinia glumarum* (Schmidt) Eriks. and Henn. was reported for the first time in the United States. It is so well known in Europe and certain Asiatic countries that its discovery at so many widely distant points in the western states led to considerable speculation as to time and method of introduction into America. Although we are still lacking definite information on these points, it is now definitely known that an examination of herbarium specimens at the New York Botanic Gardens indicates the fact that *P. glumarum* was collected in this country as long ago as June, 1892, when

C. V. Piper reported it as *P. rubigo-vera* on *Elymus americanus* and a month later as *P. rubigo-vera* on *Bromus hookerianus*, and distributed it under numbers 41 and 206. These specimens were found at Seattle and Everett, Washington, respectively.

Other American collections of *Puccinia glumarum*, made prior to 1915, were by E. T. and E. Bartholomew in August, 1913, at Billings, Mont., on *Hordeum jubatum* under number 4369; E. Bartholomew on *Hordeum jubatum* at Rock River, Wyo., in August, 1911, under numbers 1063 and 3763, and by A. O. Garrett in 1907 and 1909 in Utah and distributed as *P. rubigo-vera* under numbers 138, 191, and 192.

It would thus appear that *Puccinia glumarum* has been present in America at least twenty-five years and possibly longer.

H. B. HUMPHREY

Newton B. Pierce. The death is announced on October 13, 1916, at the age of sixty years, of Mr. Newton B. Pierce, formerly pathologist in the Bureau of Plant Industry.

Mr. Pierce in his early manhood was engaged in the lumber business, in partnership with his brother at Ludington, Mich. He was from a boy interested in natural history and spent a great deal of his time in the woods. At first he turned his attention to economic entomology, and very early developed into an excellent entomological artist. He was specially gifted in the field of draughtsmanship, particulary in the delineation of plants and insects. Desiring to advance his knowledge along entomological lines he went to Harvard and took up some special work in entomology. Conditions were not favorable for the best work, and he relinquished his entomological studies, and, after casting about, decided to take up work in plant pathology under Dr. Volney Spaulding, of the University of Michigan. Largely through Dr. Spaulding's influence, Mr. Pierce became intensely interested in plant pathological studies. He developed into a keen observer and a thorough-going investigator.

In these early days there was comparatively little pathological work carried on in this country, and those engaged in it were few in number. The pathological work of the government had only just been inaugurated.

About 1887 or 1888 there appeared in California a serious grape disease, which spread rapidly and caused immense damage to the vine industry of that state. Early in 1889 the disease had become so virulent that the Section of Vegetable Pathology in the U. S. Department of Agriculture decided to undertake an investigation of the trouble. In casting about for someone to take up this work Professor Spaulding of Michigan was communicated with. He recommended Mr. Pierce, and Mr. Pierce was appointed. Mr. Pierce proceeded at once to Santa Ana, Calif., and made

that place his headquarters. He began at once a careful field study of the disease. After six or eight months of field studies he decided that he wanted to go abroad in order to get a line on the diseases of the grape in the south of France and in Italy. He went abroad at his own expense, and was away six or eight months. Upon his return he renewed his investigation of the grape diseases, and eight or nine months later published his valuable report on the California vine disease. Mr. Pierce continued grape investigations and took up other lines of work.

Gradually the laboratory at Santa Ana grew into one of the most important branches of the plant pathological work of the government. The name was changed to the Pacific Coast Laboratory, and Mr. Pierce was put in charge. He remained in charge of this work until December 31, 1906, when he resigned.

During Mr. Pierce's work on the Coast he conducted important investigations in the California vine disease, leaf curl of the peach, walnut blight, and the diseases of the grape.

Mr. Pierce was a man of quiet and unassuming habits. He was preeminently an investigator, and preferred to work alone. A careful study of the record of his accomplishments on the Pacific Coast will show that he was an indefatigable and earnest student.

B. T. GALLOWAY

Pathological greenhouse. On the basis of the presentation in PHYTOPATHOLOGY for February, 1916, of the need for controlled greenhouse conditions in botanical research (Potter, Alden A. The control of experimental conditions in phytopathological research, p. 81) funds have been secured for carrying out these ideas in connection with the investigations of the Bureau of Plant Industry on the cereal rusts, and it is hoped to have at least one unit of this apparatus in operation in Washington within the present year.

Cereal rust survey. An extensive barberry and cereal rust field survey is projected for the coming spring and summer by the Office of Cereal Investigations, Bureau of Plant Industry.

Personals. Dr. J. L. Weimer, formerly assistant in the Department of Botany of Purdue University, Lafayette, Indiana, has been appointed scientific assistant in the Bureau of Plant Industry, effective February 14, to take up work on the diseases of sweet potatoes and other truck crops.

Mr. Moses Levine, assistant in the Department of Botany and Plant Pathology, University of Minnesota, has recently been appointed assistant in Plant Pathology in the Kansas Agricultural College, Manhattan, Kan.

REPORT OF THE EIGHTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The eighth annual meeting of the Society was held in Barnard College, Columbia University, New York City, N. Y., December 27–30, 1916, in conjunction with the American Association for the Advancement of Science.

About ninety members were present and a program of eighty-three papers was presented, the abstracts of which appeared in the last number of PHYTOPATHOLOGY. Twenty-four new members were elected, making a total of three hundred and sixty-seven.

Joint sessions were held with Section G of the American Association for the Advancement of Science and also with the Botanical Society of America.

The following officers were elected:

President, Dr. Mel. T. Cook, Agricultural Experiment Station, New Brunswick, N. J.

Vice-President, Dr. Charles Brooks, U. S. Department of Agriculture, Washington, D. C.

Councilor for three years, Prof. H. S. Jackson, Purdue University Agricultural Experiment Station, Lafayette, Ind.

One of the Chief Editors of PHYTOPATHOLOGY *for three years*, Dr. G. P. Clinton, Agricultural Experiment Station, New Haven, Conn.

Associate Editors, J. B. Rorer, Port-of-Spain, Trinidad; Prof. H. P. Barss, Oregon Agricultural College, Corvallis, Ore.; Dr. Geo. M. Reed, University of Missouri, Columbia, Mo.; and Dr. H. A. Edson, U. S. Department of Agriculture, Washington, D. C.

The Society decided to hold its next annual meeting at Pittsburgh, Pa., in conjunction with the American Association for the Advancement of Science, December 28, 1917, to January 2, 1918.

AMENDMENT TO THE CONSTITUTION

Article II, Section 1, line 1, after the word "include" the word *sustaining* was inserted.

Article III, Section 3, line 1, after the word "become" *a sustaining life member by paying one hundred dollars in ten consecutive annual payments was substituted for* "a life member upon the payment of fifty dollars."

REPORTS OF COMMITTEES

The Committee on Common Names, consisting of F. C. Stewart, G. P. Clinton, F. L. Stevens, E. C. Stakman, and W. A. Orton, presented the following report and recommendations:

Carrying out the instructions of the Society at the Columbus meeting, the Committee sent copies of the partial list of common names which it had prepared to about 200 members of the Society for their criticisms. In consequence of the suggestions received the Committee has made some changes in the list and 30 names have been stricken from the list. The revised list contains the names of 17 host plants (alfalfa to cabbage) and 92 diseases. The adoption of this list is recommended.

The Committee recommends further that this work on common names be continued under the following plan:

(1) The American Phytopathological Society shall officially adopt a list of common names of plant diseases.

(2) There shall be a permanent standing committee of the Society, called the Committee on Common Names of Plant Diseases, consisting of five members, one member being elected by the Council each year to serve five years. The Committee shall elect its own officers. The members of the present Committee (1916) shall retire in the order of the length of service of each. A vacancy on this Committee shall be filled temporarily by appointment of the President of the Society, the appointee to serve until the next annual meeting, at which time the Council shall elect a member to complete the unfinished term.

(3) This Committee shall prepare and present to the Society for official action at the regular annual meeting a list of common names of plant diseases, and at each succeeding annual meeting a supplementary list of names may be presented. At least six weeks before the annual meeting the Committee shall submit a preliminary list of names to all members of the Society for suggestions and criticism in writing. At the beginning of the annual meeting the revised list shall be conspicuously posted to invite further suggestions and criticisms. The list as finally revised by the Committee shall be presented to the Society at the same annual meeting for final adoption. The list of names officially adopted at each annual meeting shall be printed in PHYTOPATHOLOGY in the report of the meeting of the Society. The official list may be amended under the same procedure.

Upon motion the report was accepted and the recommendations adopted. Later, in connection with a discussion of the proposed list of common names which the Committee had prepared, a motion to reconsider the action in regard to the report of the Committee was adopted, and a motion was made and carried that the Committee be instructed to submit to the entire membership of the Society by mail the list of common names which it approved and to request a vote for or against each name proposed, a three-fourths majority of the total membership being necessary for adoption of any name. A motion was also adopted authorizing the Committee on Common Names to change the above plans to make them accord with the above motion.

C. W. Edgerton was appointed on the Committee in place of G. P. Clinton, whose term expired. Since the close of the meeting F. C. Stewart and F. L. Stevens have resigned and the President has appointed in their places G. R. Lyman and J. B. S. Norton.

The Committee on Ways and Means, consisting of L. R. Jones, C. L. Shear, C. W. Edgerton, H. S. Jackson, and J. T. Barrett, did not report, the Chairman being absent.

The Committee on Bibliography, consisting of L. R. Jones, C. L. Shear, and R. A. Harper, made no formal report. C. L. Shear stated that the Committee had been unable, after two years' effort, to secure the fifty subscribers necessary for publication of the proposed card index to phytopathological literature. It had been the expectation of the Committee that a large proportion of the agricultural colleges and experiment stations would subscribe for the index, but only ten such subscriptions had been obtained. The informal report was accepted and the Committee discharged.

The Committee on the Schweinitz Collection of Fungi, consisting of C. L. Shear, J. C. Arthur, and A. G. Johnson, made a report of progress to the effect that the Curator of the Herbarium had offered to take whatever steps were feasible to accomplish the purpose desired by the Society. It is hoped to perfect the details and have the matter satisfactorily attended to during the present year.

The Committee on Institutional Standardization, consisting of H. S. Reed, H. H. Whetzel, and H. R. Fulton, presented no report and requested that it be discharged. The request was approved by the Society.

The Committee on Summer Meetings, consisting of Paul Murphy, E. C. Stakman, and Donald Reddick, presented no report, the Chairman being absent.

The Committee on Pure Culture Supply Laboratory, consisting of C. L. Shear, L. R. Jones, and G. P. Clinton, made a report of progress, and stated that an item had been introduced in the appropriation bill for the Bureau of Plant Industry for the next fiscal year to cover the inauguration of this work. Whatever is accomplished in this direction during the year will depend upon the fate of this appropriation.

TREASURER'S REPORT

Receipts:			
Balance from 1915		$655.64	
Dues of—			
268 regular members for 1916	$804.00		
72 sustaining life members	720.00		
9 members for 1915	27.00		
1 sustaining life member	100.00		
Overpayment 3 members	9.00	1,660.00	
Exchange from 8 members		1.22	
Interest		15.64	
Excess transfer from Society funds		5.00	$2,337.50
Expenditures:			
Appropriation to PHYTOPATHOLOGY 1915, and 1916		400.00	
Clerical work (inc. $14.80 for 1915)		73.43	
Printing abstracts, stationery, etc		185.07	
Secretary's traveling expenses to attend Columbus meeting		44.28	
Mimeograph work for F. C. Stewart		15.00	
Supplies		7.75	
Exchange on checks		.40	
Excess dues from 3 members		9.00	
Rebate for No. 1, Vol. V, exhausted		.50	
Sinking fund for PHYTOPATHOLOGY		501.58	
Telegrams		1.55	
Transfer to PHYTOPATHOLOGY acct. members		969.00	2,207.56
Balance			$129.94

FINANCIAL STATEMENT OF BUSINESS MANAGER OF PHYTOPATHOLOGY

Receipts:			
Balance from 1915		$47.91	
Advertising guarantee 1915		150.00	
Subscriptions and sales PHYTOPATHOLOGY		620.38	
Annual dues transferred account—			
9 members for 1915	$ 18.00		
268 regular members, 1916	536.00		
72 sustaining members (1 excess)	360.00		
1 sustaining member in full	50.00		
1 member who paid $10	5.00	969.00	

Sales PHYTOPATHOLOGY direct		18.10	
Neiberg subscription (sent to W. & W.)		3.25	
Extra illustrations in PHYTOPATHOLOGY, O'Gara and Hotson		11.28	
Annual appropriation from American Phytopathological Society for 1916		200.00	
Interest on deposits		.84	
Interest on mortgage, 6 months		15.00	$2,035 76
Expenditures:			
Cooke portrait		$26.00	
Separates from PHYTOPATHOLOGY		41.95	
Stationery, express, postage (Dr. Reddick)		35.00	
Phoenix files		.57	
Illustration of horse-chestnut		10.00	
Readjustment of dues one member paid to publishers		3.00	
Readjustment Neiberg subscription, paid to treasurer		3.25	
Insurance on stored stock		5.40	
Manufacture of PHYTOPATHOLOGY:			
No. 6, Vol. V, balance from 1915	$65.80		
No. 1, Vol. VI	432.82		
No. 2, Vol. VI	348.52		
No. 3, Vol. VI	338.78		
No. 4, Vol. VI	260.92		
No. 5, Vol. VI	165.82		
No. 6, Vol. VI, $147.30, pending	000.00	1,612.66	
Williams & Wilkins miscellaneous bills for postage, etc.		78.98	
Clerical work		70.43	
Reimbursement American Phytopathological Society account over-transfer		5.00	1,892 24
Balance			$143.52

These accounts were referred to an auditing committee, consisting of A. D. Selby, R. Kent Beattie, and E. C. Stakman. The Committee reported that they had examined the accounts and found them correct, and the reports were adopted.

RESOLUTIONS ADOPTED

A Committee, consisting of H. A. Edson, A. D. Selby, and John L. Sheldon, was appointed by the Society to draft resolutions in regard to the deaths of two members, W. A. Martin and Yungyen Young, and also of Professor T. J. Burrill. The following resolutions were presented:

"*Resolved*, That whereas in the death of W. A. Martin, of Houlton, Maine, and Yungyen Young, of Shanghai, China, the American Phytopathological Society has been deprived of two of its members, the Society records its sincere regret at the taking of these gentlemen.

"That whereas in the death of Professor T. J. Burrill, of the University of Illinois, who first demonstrated the existence of bacterial diseases of plants, there has been removed an eminent leader in botanical and phytopathological research, the American Phytopathological Society expresses its sincere regret at the departure of this eminent scholar and teacher, and records its appreciation of the service rendered our science by his researches.

"That these resolutions be filed with the records of the Society and printed in PHYTOPATHOLOGY."

The following resolution was also passed by the Society:

"*Resolved*, That the Society express its deep appreciation and gratitude to the local Committee and the members of the Department of Botany of Columbia University for the excellent facilities provided and for the many courtesies extended during the meeting.

MISCELLANEOUS BUSINESS

Upon motion the Society voted to appropriate two hundred dollars from any available funds for use in the support of PHYTOPATHOLOGY for 1917.

In response to a request from the Botanical Society of America to nominate a member of the American Phytopathological Society for the editorial board of the Botanical Society, Dr. A. G. Johnson was recommended by the Council. The Society approved of the action of the Council.

The Board of Editors made the following recommendations in regard to PHYTOPATHOLOGY, and these were approved by the Council:

(1) That no article be accepted which is written in simplified spelling, but that in the case of words which have two or more forms in good usage strict uniformity is not required, but the shorter and simpler forms are to be preferred.

(2) That PHYTOPATHOLOGY be issued monthly and include five hundred or more pages during the year, the price of the JOURNAL to members, including dues, to be four dollars and to subscribers five dollars per year, the increase in price to begin January 1, 1918.

(3) Space of one-half page or more at the end of long articles is to be used for the publication of briefer articles or notes in order to avoid wasting space.

(4) It is recommended to members of the Society that they refrain from publishing original matter in extension publications, weekly news letters, and other similar publications which are not usually preserved and permanently filed, also that such references be omitted from the list of literature.

(5) Recommended that authors of phytopathological papers which are published in proceedings of academies, horticultural societies, and other publications of limited distribution, prepare abstracts covering the original matter for publication in PHYTOPATHOLOGY.

Professor J. B. S. Norton presented a plan for "a standard chart for per cent estimates" in regard to injury and conditions of diseased plants. Upon motion this plan was referred to a Committee, consisting of L. R. Jones, V. B. Stewart, and H. B. Humphrey, for consideration and report to the Society at its next meeting.

Dr. E. W. Allen, Editor of the Experiment Station Record, in response to the resolution adopted by the Society at its last meeting requesting that titles of papers abstracted in the Record be published in full in the original language, stated that after full consideration of the matter it did not appear practicable to adopt the proposed change.

Dr. Donald Reddick, Editor of PHYTOPATHOLOGY, presented a verbal report calling attention to some of the matters discussed by the Board as reported above.

Upon motion the Society directed the Secretary to publish during the year a new membership list.

The Secretary called attention to the need of prompt notice of change of address of members, in order to avoid inconvenience and loss of copies of the JOURNAL, and unnecessary expense in correspondence.

C. L. SHEAR,
Secretary-Treasurer

REPORT OF MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

A meeting of the Pacific Division was held at the University of California, Berkeley, December 28 and 29, 1916. President J. T. Barrett, of the Citrus Experiment Station, Riverside, California, presided at the sessions, while Ralph E. Smith, of Berkeley, California, acted as secretary in the absence of W. T. Horne, of Berkeley who is spending a leave of absence in Cuba. At the business session of the Society the following officers were elected for the coming year:

President, H. P. Barss, Corvallis, Oregon.

Vice President, James McMurphy, Leland Stanford Junior University, Palo Alto, California.

Secretary-Treasurer, W. T. Horne, Berkeley, California.

The following papers were presented:

Apple rosette. M. A. Willis

No abstract.

An Alternaria blight of tomatoes in California. Bruce Douglas

No abstract.

Sour rot of lemons. Clayton O. Smith

No abstract.

Stem-end decay of Valencia oranges in transit. Clayton O. Smith

No abstract.

Some effects of sulphur on soils. H. S. Reed

Sulphur in the form of elemental sulphur, sulphides, or sulphates is widely used as a fungicide. Much of this material finds its way eventually into the soil. Its effect as a soil constituent is therefore pertinent.

Under anaerobic conditions microorganisms may reduce sulphates to sulphites or sulphides, both of which are toxic to vegetation. Oxidation processes may convert sulphides and elemental sulphur to sulphates. The process is largely, if not entirely, due to biological agencies. If the oxidation process is incomplete sulphites may be formed.

The harmful effects of sulphur are more common in soil deficient in organic matter, or in soils having an acid reaction.

Black-heart disease of the apricot. Helen Czarnecki

Studies on Monilia. Edith Phillips

No abstract.

Miscellaneous observations. James McMurphy

No abstract.

The experimental investigation of alleged smelter smoke injury in Calaveras County, California. W. W. Thomas

No abstract.

Pythiacystis related to Phytophthora. J. T. Barrett

For some time three strains, perhaps distinct species, of a fungus, whose asexual stages resemble very closely those of *Pythiacystis citrophthora* Smith & Smith, have been under observation. These strains differ mainly from the latter fungus in that

they produce in culture oospores while the perfect stage has not yet been reported from any culture of *P. citrophthora* isolated from any variety or species of Citrus.

Of the three strains mentioned, one was isolated from decaying apples in March 1908 in Illinois; one from bark of a young apricot tree in March 1916 in California and the third from bark of an avocado tree by H. S. Fawcett in May 1914.

A comparison of the three forms with four species of Phytophthora has revealed a very close similarity of the oogonia, oospores, and antheridia to those of *P. cactorum* (Cohn & Leb.) Schroeter, while their asexual spores, (sporangia and conidia) differ mainly in the manner of separating from the hyphae.

This marked similarity of their sexual organs to those of *P. cactorum*, and of their sporangia to those of *Pythiacystis citrophthora* would seem to indicate a close relationship of the two genera Pythiacystis and Phytophthora.

Variations in Colletotrichum glœosporioides. O. F. BURGER

Cultures of *Colletotrichum glœosporioides* were isolated from different *Citrus* species in California. They have been grown on six different media and each strain responds differently to the media. The size of the spore, depends in part upon the medium used. A hundred spores of each strain were measured and it was found that the mean spore-length of most of the strains, when grown on green bean pods, is 15 microns. Other strains were found, however, which have a mean spore-length of 12 and 17 microns respectively.

The cultures can be classified according to their mycelial characters, when grown on artificial media. Class A. Mycelium dark, olive color, giving a fluffy growth with but scant spore production. Class B. Mycelium dark, appressed but abundant spore production. Class C. White mycelium and abundant spore production.

Sexuality in Cunninghamella. O. F. BURGER

Pure cultures were made from single spore heads of *Cunninghamella bertholletiæ* and during the entire work no zygospores were formed in the culture tubes. But when two strains, whose gametes were compatible are contrasted in an agar plate zygospores are produced at a point where the cultures meet.

A sexual reaction did not occur with Blakeslee's Mucor V, plus and minus, or his plus and minus strains of *Cunninghamella echinulata.* The strains of *C. bertholletiae* which acted as neutrals with these two fungi formed normal zygospores when contrasted among themselves.

The peculiarity in their method of conjugation is, that there were marked differences in their ability to conjugate with certain strains. Strain *A* will conjugate with strains *B* and *C* and strains *B* and *C* will also conjugate and form normal zygospores. *Cunninghamella bertholletiæ* is therefore a pseudo-heterothallic mucor.

Curly top of the sugar beet. RALPH E. SMITH

No abstract.

One session was devoted to a discussion of the so-called non-parasitic or physiological plant diseases, attention being paid to a number of obscure troubles which are of particular importance in the Far West. A discussion also took place concerning possible means of making the Society more useful and securing a representative attendance from the different states at its meetings. The acting secretary was instructed to take up the latter subject with members in the territory covered by the Division to see what can be done along this line.

W. T. HORNE,
Secretary-Treasurer

LITERATURE ON AMERICAN PLANT DISEASES[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY AND FLORENCE P. SMITH, ASSISTANT

December, 1916, to January, 1917

Alaska Agricultural Experiment Stations. Plant diseases. Alaska Agr. Expt. Stas. Rpt. **1915**: 39–41. 1916.

Allard, Harry Ardell. A specific mosaic disease in Nicotiana viscosum distinct from the mosaic disease of tobacco. Jour. Agr. Research **7**, no. 11: 481–486, pl. 35. December 11, 1916.

Averna Saccá, Rosario. Molestias cryptogamicas do cafèeiro. Bol. Agr. [São Paulo] **17**, no. 10: 790–840, fig. 157–285. Outubro, 1916.

Baker, Carl Fuller. Additional notes on Philippine plant diseases. Phil. Agr. and Forester **5**, no. 3: 73–78. July, 1916.

Bancroft, C. Keith. Report on the South American leaf disease of the Para rubber tree. Jour. Bd. Agr. Brit. Guiana **10**, no. 1: 13–33. October, 1916.

Fusicladium macrosporum; die back.

Boyce, J. S. Pycnia of Cronartium pyriforme. Phytopathology **6**, no. 6: 446–447. December, 1916.

On *Pinus ponderosa.*

Brandes, E. W. Report of the plant pathologist. Porto Rico Agr. Expt. Sta. Rpt. **1915**: 34–35. 1916.

Experiments in the control of a banana disease; miscellaneous experiments.

Brooks, Charles, and Cooley, Jacquelin Smith. Temperature relations of apple-rot fungi. Jour. Agr. Research **8**, no. 4: 139–164, 25 fig., 3 pl. January 22, 1917.

Chivers, Arthur Houston. An epidemic of rust on mint. Mycologia **9**, no. 1: 41–42. January, 1917.

Puccinia Menthæ Pers.

Cook, Melville Thurston, and Wilson, Guy West. The influence of the tannin content of the host plant on Endothia parasitica and related species. New Jersey Agr. Expt. Sta. Bul. 291, 47 p. 1916.

Shorter article with same title published in Bot. Gaz., November, 1915. Noted in previous list.

Dodge, Bernard Ogilvie, and Adams, James Fowler. Notes relating to the Gymnosporangia on Myrica and Comptonia. Mycologia **9**, no. 1: 23–29, 1 fig., 3 pl. January, 1917.

[1] This list aims to include the publications of North and South America, the West India Islands, and islands controlled by the United States, and articles by American writers appearing in foreign journals. All authors are urged to coöperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

Elliott, John A. The sweet potato "soil rot" or "pox" organism. Science n. s. **44**, no. 1142: 709–710. November 17, 1916.
Cystospora batata gen. nov., sp. nov.

Fawcett, George L. A Porto Rican disease of bananas. Porto Rico Agr. Expt. Sta. Rpt. **1915**: 36–41. 1916.
Closely resembles Panama disease.

Fawcett, Howard S. Citrus scab. Phytopathology **6**, no. 6: 442–445. December, 1916.
Discussion of article by Grossenbacher.

——— A Pythiacystis on avocado trees. Phytopathology **6**, no. 6: 433–435. December, 1916.

Fromme, Fred Denton, and Thomas, H. E. The root-rot disease of the apple in Virginia. Science n. s. **45**, no. 1152: 93. January 26, 1917.
Xylaria sp.

Germano de Souza, Raul. O Tylenchus acuto-acudatus (?) nos cafezaes de Indaiatuba. Bol. Agr. [São Paulo] **17**, no. 9: 726–736, 7 fig. Setembro, 1916.

Gile, Philip Lindsey. Chlorosis of pineapples induced by manganese and carbonate of lime. Science n. s. **44**, no. 1146: 855–857. December 15, 1916.

Güssow, Hans Theodor. Canada's white pine possessions are threatened with extermination. An authoritative discussion of white pine blister rust. Canad. Forestry Jour. **13**, no. 1: 900–906, illus. January, 1917.

——— Leaf roll in tomatoes? Phytopathology **6**, no. 6: 447. December, 1916.

Hartley, Carl, and Pierce, Roy Gifford. The control of damping-off of coniferous seedlings. U. S. Dept. Agr. Bul. 453, 32 p., 1 fig., 2 pl. 1917.
Pythium debaryanum; Fusarium moniliforme; Corticium vagum var. Solani.

Heald, Frederick De Forest. Some new facts concerning wheat smut. Proc. Washington State Grain Growers, Shippers and Millers Assoc. 10th Ann. Meeting, **1916**: 38–45, 2 fig. 1916.

Huard, Victor Amédée. Les principales espèces d'insectes nuisibles et de maladies végétales. [Quebec Dept. Agr.] Bul. 23, 75 p., 78 fig. 1916.

Hubert, Ernest E. Celluloid cylinders for inoculation chambers. Phytopathology **6**, no. 6: 447–450, 1 fig. December, 1916.

Jehle, Robert Andrew. Means of identifying citrus canker. Quart. Bul. State Plant Bd. Florida **1**, no. 1: 2–10, 12 pl. (partly col.). October, 1916.

Johnson, James. Enfermedades del tabaco y manera de combatirlas. Parte V. Hacienda **12**, no. 4: 124–126, illus. Enero, 1917.

Johnston, John Robert. Enfermedad de la raiz de la caña de azúcar. Hacienda **12**, no. 4: 117–118, illus. Enero, 1917.
To be continued.

——— Marasmius on sugar cane. Mycologia **8**, no. 2: 115. March, 1916.
Comparison of *M. stenophyllus* and *M. sacchari*.

Lewis, A. C. Cotton wilt in Georgia. Georgia State Bd. Ent. Bul. 40, 18 p., 8 pl. 1915.
Varieties of wilt resistant cotton, p. 8 ff.

McClintock, James A. Is cucumber mosaic carried by seed? Science n. s. **44**, no. 1144: 786–787. December 1, 1916.

——— Peanut mosaic. Science n. s. **45**, no. 1150: 47–48. January 12, 1917.

——— Sclerotinia libertiana on snap beans. Phytopathology **6**, no. 6: 436–441, 2 fig. December, 1916.

McCubbin, W. A. The white pine blister rust: does the fungus winter on the currant? Science n. s. **45**, no. 1152: 87. January 26, 1917.

Martin, William H. Influence of Bordeaux mixture on the rates of transpiration from abscised leaves and from potted plants. Jour. Agr. Research **7**, no 12: 529–548. December 18, 1916.
Literature cited, p. 547–548.

Massey, L. M. The hard rot disease of gladiolus. New York Cornell Agr. Expt. Sta. Bul. 380: 149–181, fig. 38–44, pl. 15–16. 1916.
Bibliography, p. 180–181.
Septoria Gladioli.

Mendiola, Nemesio, and Espino, Rafael B. Some phycomycetous diseases of cultivated plants in the Philippines. Phil. Agr. and Forester. **5**, no. 3: 66–71, illus. July, 1916.
Bibliography, p. 71.
Phytophthora diseases; *Pythium Debaryanum*.

Mix, A. J. Cork, drouth spot and related diseases of the apple. New York State Agr. Expt. Sta. Bul. 426: 473–522, 12 pl. 1916.
Nonparasitic.

——— Sun-scald of fruit trees, a type of winter injury. New York Cornell Agr. Expt. Sta. Bul. 382: 233–284, fig. 60–61, pl. 18–19. 1916.
Literature cited, p. 283–284.
Probably a winter injury caused by direct freezing to death of the tissue.

Morse, Warner Jackson. Studies upon the blackleg disease of the potato, with special reference to the relationship of the causal organisms. Jour. Agr. Research **8**, no. 3: 79–126. January 15, 1917.
Literature cited, p. 124–126.
Bacillus atrosepticus Van Hall.

Nowell, William. Fungoid and bacterial diseases. West Indian Bul. **15**, no. 2: 133–143, 146 147. 1915.
Listed under host and locality.
See also p. 121–122.

——— Fungous and bacterial diseases. West Indian Bul. **16**, no. 1: 17–25, 28–29. 1916.
Listed under host and locality.
See also p. 2.

——— Rosellinia root diseases in the Lesser Antilles. West Indian Bul. **16**, no. 1: 31–71, 12 fig. on 4 pl. 1916.
References, p. 69–71.

Orton, Clayton Roberts. Phytophthora infestans on tomatoes in Australia. Phytopathology **6**, no. 6: 447. December, 1916.

Osner, George A. Leaf smut of timothy. New York Cornell Agr. Expt. Sta. Bul. 381: 185–230, fig. 45–58, pl. 17. 1916.
Bibliography, p. 226–230.
Ustilago striaformis.

Paul, B. H. The pine blister rust. Conserv. Com. State New York Bul. 15, 18 p., illus., 1 fold. map. [1917.]

Seaver, Fred Jay. Some papers presented during convocation week. Mycologia **9**, no. 1: 42–46. January, 1917.
Joint session of Bot. Soc. and Phytopath. Soc., Friday, December 28, 1916, in connection with Amer. Assoc. Adv. Sci.

Smith, Erwin Frink. Mechanism of tumor growth in crowngall. Jour. Agr. Research **8**, no. 5: 165–186, pl. 4–65. January 29, 1917.
Literature cited, p. 185–186.

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PHYTOPATHOLOGY

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STUDIES ON BACTERIUM SOLANACEARUM[1]

E. E. STANFORD AND F. A. WOLF

WITH ONE FIGURE IN THE TEXT

Studies on the wilt diseases caused by *Bact. solanacearum* have been in progress at the North Carolina Agricultural Experiment Station since 1903. A recent bulletin (4) dealing primarily with remedial and palliative measures for tobacco wilt contains the results of certain of these investigations. In the present paper are presented data bearing (1) on the distribution within North Carolina of the disease on tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*), (2) on cultural studies to determine the identity of the strains from the several hosts and (3) on the results of cross inoculations.

DISTRIBUTION OF BACT. SOLANACEARUM WITHIN NORTH CAROLINA

Tobacco wilt has probably been present within the state for at east twenty-five years, but occurs in only a small proportion of the lands devoted to tobacco culture. The disease was first definitely recognized in the southern portion of Granville county in 1902. Records indicate that the disease has yearly continued to spread so that it now occurs in eleven counties, namely: Granville, Durham, Vance, Wake, Franklin, Ashe, Guilford, Greene, Davidson, Chatham and Yadkin.

The losses in the first four of these counties are confined to the southern portions of Granville and Vance and the northern portions of the adjacent counties of Durham and Wake. In this area the disease is so generally destructive that the growing of tobacco has been abandoned on many farms. The disease is confined, however, to relatively small areas in the seven remaining counties.

[1] Acknowledgment is hereby made to the several members of the Department of Botany and Plant Pathology of North Carolina Agricultural College and Experiment Station, who, since the inception of these investigations, have aided in the work.

It is a striking fact that collections of bacterial wilt of tomato which have been very generally made throughout the State show that the disease on tomatoes occurs in thirty-nine counties of the state. It is realized, of course, that the disease on both tobacco and tomatoes may not have been reported from all localities in which it may occur, yet the fact remains that the disease on the two hosts is not coextensive. No adequate explana-

FIG. 1. MAP OF NORTH CAROLINA SHOWING THE KNOWN DISTRIBUTION OF BACTERIUM SOLANACEARUM ON TOBACCO AND ON TOMATO

tion is at hand to account for this, in view of the fact that reciprocal inoculations are so easily effected and that the strains of *Bact. solanacearum* from tobacco and tomatoes are identical as shown by the cultural studies of several investigators and confirmed by studies to be presented later in this paper.

It might be added, that no data have accumulated relative to the distribution of this parasite on other solanaceous hosts and that the disease on peanut (*Arachis hypogæa*) has been observed in one locality only.

CULTURAL STUDIES

In view of the fact that in certain sections, *Bact. solanacearum* is not present on all of the hosts which are known to be subject to attack, a study by means of artificial cultures was made to determine the identity of the strains from the more common hosts. Several investigators, among whom may be mentioned Smith (5 and 6) and Honing (6)[1] have previously reported extensive studies on Bact. solanacearum in artificial culture and regard the organism from the several hosts as identical. Nevertheless,

[1] References to the publications of Honing, Hunger, and Uyeda are given in Volume 3 of Smith's Bacteria in Relation to Plant Diseases. The authors have not seen these publications, but only the abstracts given by Dr. Smith. Bibliographies of *Bact. solanacearum* on p. 218-219 and 270-271.

parallel cultures of the organism isolated from tobacco, potato, (*Solanum tuberosum*) tomato, eggplant (*Solanum melongena*), peanut, ragweed (*Ambrosia artemisiifolia*) and *Eclipta alba* have been made. These isolations were made from various parts of the hosts and from plants from several localities within the state. In general, the cultural variations which appeared between the strains from the different hosts were no greater than those from strains all of which came from the same host. Since, however, certain additiona' facts are brought to light, a brief account of the growth in culture is pertinent and is herewith given.

Colonies on agar plates usually become visible within thirty-six to forty-eight hours at 28°C. At a temperature 4 to 5° lower, they may be scarcely noticeable at the end of seventy-two hours. They are at first white, wet-shining and opalescent, circular in outline, slightly raised and with smooth margin. Colonies become 3 to 5 mm. in diameter in five to six days. They soon become distinctly opalescent-blue by transmitted light, when the plates are held some distance from a poorly lighted reflecting surface. When the colonies are viewed with the blue sky for a background, they are distinctly brown with faint concentric rings. Later, they become very markedly brown by reflected light. The pigmentation originates at the center of the colony, spreads toward the margin and is diffused somewhat into the agar. The color is more intense at the center of the colony. Subsurface colonies are globose or lenticular, much smaller than the surface colonies and become brown much sooner than do the surface colonies.

The growth on agar slants develops slowly, is filiform at first and usually spreading at the bottom of the slant. It is slightly elevated and the margin is entire or wavy. Colors and pigmentation develop similar to those in plate colonies with considerable variation in time of appearance and intensity of pigmentation which may vary from scarcely perceptible to brownish black. When Witte's peptone is used, a deeper pigmentation develops than when Difco peptone is employed. When the colonies become blackened, the organism is no longer viable. It appears to retain its vitality for about four and one-half months on agar but rapidly loses its virulence on this medium.

The appearance of colonies on litmus lactose agar slants is similar to those on nutrient agar. The litmus is at length slightly reduced. Pigmentation is first noticeable in three to four weeks and a brown stain soon becomes diffused throughout the agar and masks its color. Portions of the substratum which are not brown become sky-blue by diffuse light and reddish plum-colored by transmitted light.

The appearance of this organism in bouillon cultures is somewhat variable. A rather uniform clouding develops within twenty-four hours

which becomes so intensely opaque within a week as to make it impossible to see through a bouillon tube when the tube is placed immediately in front of an object. Numerous pseudogloeae may appear forming flocculent particles on the surface. No pellicle nor ring is formed, but a thin, opalescent scum appears on the surface. A dirty white, viscous precipitate develops within a week or two. Within four to six weeks, this precipitate will have become dense and the supernatant bouillon will have become clear at which time the organism is no longer viable. Various degrees of pigmentation may occur, being more intense with Witte's than with Difco peptone. Tubes of the bouillon made with the former become brownish black on long standing.

On potato plugs, the growth is spreading, thin or slightly raised, white or flesh-colored at first but rapidly becomes brown, often pitch-black. The surrounding liquid becomes clouded and brown. The organism is short-lived on this medium and commonly loses its vitality within a week. The virulence of *Bact. solanacearum* on artificial media is best retained on potato plugs, but transfers must be made at intervals of about two days.

When grown on milk, there is no peptonization nor precipitation of casein. A slight viscidity and the odor of putrefaction are developed and the medium at length becomes brown and alkaline.

The organism causes a partial clarification of litmus milk with a deepening of the blue color which appears reddish by transmitted light. A slight dirty white precipitate is formed, which becomes brown on long standing. The bacteria may remain viable for five months on this medium.

The surface colonies on gelatin are small, circular, white and wet-shining. Submerged colonies are globose and yellowish to brownish. Growth along the line of the stab on gelatin is white, later becoming brown, filamentous and best at the surface of the medium. No liquefaction occurs.

There is no evident growth on Cohn's solution in four weeks. On Uschinsky's solution, growth ranges from none to feeble with slight clouding.

In dextrose broth, a copious growth develops in the open arm, extending only to the base of the closed arm. An abundant, rather viscous precipitate appears. The medium at length becomes brown in the open arm and is strongly alkaline with no evolution of gas.

Cultural characters on saccharose broth are similar to those on dextrose with a less marked tendency to the development of a brown color.

Growth is feeble on lactose broth with little sedimentation and little or no brown color is developed, even after seven weeks. The acidity of acid broth is diminished but neutral broths are not rendered alkalin. On mannit, the growth characters are similar to those on lactose. A very

copious growth ensues in glycerin broth with a marked development of brown color. Growth on maltose is similar to that on dextrose. Nitrates and ammonia are formed in moderate amount in nitrate solutions.

Growth is much delayed and diminished in hydrochloric acid +25 Fuller's scale, and is entirely inhibited at +30. No growth occurs in double strength bouillon rendered +33 acid by the addition of expressed tomato fruit juice. Smith (6) reports growth in +33 acid of beef juice. The optimum reaction lies between +10 and +15.

Growth is slight or none in bouillon −5 with sodium hydroxid. No growth occurred in −10 sodium hydroxid. The organism is little retentive of vitality on culture media. Milk appears to be the best medium for long continued growth on *Bact. solanacearum*. The organism may remain viable for two months in sterilized distilled water. No evidence of diastatic activity was found when the organism was grown on potato plugs.

A considerable number of special media have been prepared, among which are soil extract, casein agar, Heyden's Nährstoff agar, potato agar, potato leaf agar, and tomato leaf agar. No growth of diagnostic significance developed on any of these media.

Bacterium solanacearum is very short-lived in mixed cultures. Honing (6) noted a marked antibiosis between the wilt organism and *B. mesentericus* as well as other species plated from wilted tobacco. In our studies also, various bacteria have been found to replace *Bact. solanacearum* in decaying, wilted plants. Five strains of yellow chromogens isolated from diseased tomatoes, tobacco and peanuts were found in the fall of 1915 to exhibit marked antagonism to the wilt organism. In intersecting streaks on agar plates, the chromogens tended to crowd out the parasite. The presence of *B. mesentericus* and other soil inhabitants appear never to be so antagonistic, however, as to eliminate *Bact. solanacearum* from infested soils.

Thus far attempts to isolate the parasite directly from infested soils have been unsuccessful. This is due in part at least to the fact that *Bact. solanacearum* is inhibited by other soil inhabitants which develop on the plates. Honing (6) however, succeeded in isolating it on plates from dilution cultures of well water.

CROSS-INOCULATION EXPERIMENTS

Bacterium solanacearum has previously been shown to attack members of eight widely separated families, Urticaceæ, Leguminosæ, Tropaeolaceæ, Euphorbiaceæ, Verbenaceæ, Solanaceæ, Pedeliaceæ and Compositæ.

The organism was first described by Erwin F. Smith (5) in 1896 as

the cause of a wilt disease of tomato, eggplant and potato and he successfully inoculated *Solanum nigrum*, *Datura stramonium*, *D. metelloides*, *D. fastuosa*, *D. cornucopia*, *Physalis crassifolia*, *P. philadelphica* and *Petunia* (hybrid).

Several investigators, among whom are Hunger (6), Stevens and Sackett (7) and Uyeda (6) have reported a wilt disease of tobacco. Honing (6) in 1910 first reported this organism as the cause of disease in plants outside of the Solanaceæ. He found it in *Pouzolzia* sp., *Physalis angulata*, *Indigofera arreeta*, *Arachis hypogœa*, *Mucuna* sp., *Acalypha boehmeroides*, *Ageratum conyzoides*, *Spilanthes acmella*, *Pluchea indica*, *Blumea balsamifera*, *Synedrella nodiflora* and *Tectona grandis*. He also successfully inoculated several ornamental varieties of *Nicotiana*, *Capsicum annuum* and *Sesamum orientale*.

A wilt disease upon peanut was subsequently reported from North Carolina (2) and later studies (3) in this state added two composites, *Ambrosia artemisiifolia* and *Eclipta alba*, to the list of naturally infected hosts.

A wilt of nasturtium (*Tropœolum majus*) caused by *Bact. solanacearum* was reported from Maryland by Bryan (1). She succeeded in inoculating also the common cultivated Ageratum and Verbena.

The artificial inoculation experiments conducted at the North Carolina Experiment Station prior to 1913 were confined primarily to solanaceous plants. When in the summer of 1912, it was found that peanuts are subject to attack by *Bact. solanacearum*, this host was successfully inoculated with strains from tobacco, peppers and peanuts. The strains from peanuts were also found to be pathogenic to tobacco.

During the season of 1914, *Bact. solanacearum* was isolated from diseased ragweeds (*Ambrosia artemisiifolia*) and subsequently found to be productive of wilt on tobacco, tomato, potato, *Eclipta alba* and garden nasturtium (Tropœolum). The reciprocal inoculations upon ragweed with strains from tobacco, tomato, potato and *Eclipta alba* were rather unsuccessful. No systemic invasion resulting in death, but merely a local occlusion and blackening of xylem elements occurred in inoculated plants.

In the fall of 1915, strains isolated from wilted *Eclipta alba* were successfully inoculated into tomatoes, potatoes, tobacco, garden nasturtiums and *Eclipta alba*. A rather more comprehensive series of inoculations on cultivated and wild species was instituted in 1916, the results of which are herein briefly summarized.

Method of inoculation. The strain of *Bact. solanacearum* employed in making the initial inoculations was obtained by the poured plate method, from wilted tobacco plants from Creedmoor, North Carolina. As soon

as the organism had developed on these poured plates, transfers were made to potato plugs. Repeated transfers at intervals of one to three days were made on this medium. The pigmentation on old cultures on agar and on potato plugs was regarded as sufficiently characteristic to establish the identity of the wilt organism. Inoculum from one- to three-days-old cultures on potato plugs was used in all of the inoculations. Inoculations were made by pricking the plants near the tips of the branches and inserting the inoculum. A number of check plants, either uninjured or pricked with a sterilized needle were used in the case of each species tested. Since it was known that *Bact. solanacearum* loses its virulence even though repeated transfers are made, no attempt was made to use the original strain from tobacco in all of the inoculations. Instead, isolations from certain of the inoculated species were used in continuing the series of inoculations. Some differences in virulence appeared in strains which had passed through different hosts but no such marked decrease occurred as when the organism is repeatedly transferred on culture media.

In general, young, vigorously growing plants were used in these tests, although in some cases, rather mature plants were employed. The cultivated species were grown either in the greenhouse or in small experimental plats at West Raleigh, North Carolina and the weeds grew in waste places where they could be kept under observation for the necessary length of time. In general, as soon as inoculated plants showed symptoms of disease, they were examined microscopically to determine the presence of bacteria within the tissues at points remote from the point of inoculation. The organism was then reisolated by the poured plate method, and its identity established by the characteristic growth on agar and potato plugs. As supplementary evidence, the reisolated organism was inoculated into tomatoes or tobacco.

The accompanying diagram of the plan of these cross-inoculation experiments has been so arranged as to show at once the source of the inoculum, the result of the inoculation and the number of plants inoculated.

Results. When comparison is made with the host species previously enumerated, it will be seen from this tabulation of the results of cross inoculations that the following plants have heretofore been unreported as subject to attack by *Bact. solanacearum: Stizolobium niveum, Tropæolum lobbianum, T. peregrinum, Croton glandulosus* var. *septentrionalis, Impatiens balsamina, Verbena erinoides, Lycopersicon cerasiforme, L. pyriforme, Browallia demissa, Physalis alkekengi, Schizanthus pinnatus, Salpiglossis sinuata* and *Martynia proboscidea.* Twelve of these species belong to families representatives of which had hitherto been known to be subject to attack and one species, *Impatiens balsamina*, belongs to an additional family.

TABLE 1

Diagram of cross inoculations with Bacterium solanacearum[1]

		∞Amaranthus gangeticus, 10 ∞Amaranthus caudatus, 10 zIMPATIENS BALSAMINA, 20 zzSolanum carolinense, 2 z*Tropaeolum lobbianum*, 4 zTropaeolum majus, 4 ∞Helenium tenuifolium, 11 zCROTON GLANDULOSUS VAR. SEPTENTRIONALIS, 28*	∞Boehmeria cylindrica, 10 ∞Impatiens fulva, 10 ∞Stisolobium niveum, 2 ∞Physalis alkekengi, 1 zzERIGERON CANADENSIS, 18 oAcalypha virginica, 20 oEuphorbia nutans, 10 zzAmbrosia artemisiifolia, 10 zz*Solanum carolinense*, 10 zBROWALLIA DEMISSA, 22 zzStisolobium niveum, 4 oPhysalis angulata, 4		
Nicotiana tabacum	zAMBROSIA ARTEMISIIFOLIA, 30			zMARTYNIA PROBOSCIDEA, 2 oVerbena venosa, 4 ∞Petunia (hybrid), 6 ∞Datura cornucopia, 2 ∞Datura fastuosa, 6 ∞Chenopodium botrys, 3 zLYCOPERSICON CERASIFORME, 4 ∞Nierembergia gracilis, 6* ∞Phytolacca decandra, 4 ∞Sida spinosa, 5 zzBIDENS BIPINNATA, 10* zSolanum nigrum, 1 zSolanum melongena, 2 ∞Physalis alkekengi, 4 zEclipta alba, 10* zzStisolobium niveum, 2 ∞Adicea pumila, 10 ∞Boehmeria cylindrica, 10 zPHYSALIS ALKEKENGI, 6	{zLycopersicon cerasiforme, 8 z*Salpiglossis sinuata*, 2 zzStisolobium niveum, 8 {zTropaeolum peregrinum, 2 oSchizanthus pinnatus, 2 {zz*Impatiens sultana*, 1 zMartynia proboscidea, 2
	zDATURA TATULA, 10	zzSTISOLOBIUM NIVEUM, 4	zVERBENA ERINOIDES, 4 {zTropaeolum peregrinum, 2 z*Schizanthus pinnatus*, 2		

[1] All species within a brace were inoculated from cultures from the species appearing in small capital letters within the preceding brace. The wilt organism has been isolated from all species appearing in italics and successfully reinoculated into either or both tobacco and tomatoes. The character before the various species indicates the nature of the injury. *z*, signifies wilt or serious injury; *zz*, bacteria present in considerable numbers, but productive of no external evidence of injury; *o*, no injury, bacteria present only near the point of inoculation, not reisolated but the expressed ooze when used as inoculum, resulted in infections on tobacco; *o*, bacteria remain viable for a considerable time near the point of inoculation with only local injury of tissues, no reisolation; *oo*, no demonstrable infection of any sort. The numerals following the name of the hosts denote the number of plants inoculated. The asterisk (*) following the numeral indicates that strains from more than one host have been used in these inoculations with the development of similar symptoms.

It may also be noted from the tabulation that no infection resulted in *Stizolobium niveum* and *Physalis alkekengi* when inoculated with the organism isolated from wilted *Impatiens balsamina*. Further, no demonstrable infection resulted in the case of *Petunia* (hybrid), *Datura cornucopia*, *D. fastuosa*, and *Physalis alkekengi* when the isolations were made from wilted *Browallia demissa*. Smith (6) had previously shown the first three of these forms to be subject to attack. Since *Stizolobium niveum* wilted when *Datura tatula* was the source of the inoculum and *Physalis alkekengi*, when wilted *Verbena erinoides* was employed, it is indicated that virulence is influenced by the host plant.

In the following species, little or no external injury resulted from inoculation, but the vascular tissues were found to be invaded: *Euphorbia nutans*, *Solanum carolinense*, *Physalis angulata*, *Impatiens sultani*, *Bidens bipinnata* and *Erigeron canadensis*.

Inoculated plants of *Ambrosia artemisiifolia* and *Eclipta alba* wilted thus confirming previous studies (3).

In general, it can be said that the external symptoms and pathological histology of the plants which were artificially inoculated in these studies differed in no essential particular from those of other species which have previously been reported as hosts for *Bact. solanacearum*. The species of Tropæolum, Lycopersicon, Browallia and Eclipta tested are to be regarded as very susceptible, whereas, *Stizolobium niveum* and *Physalis alkekengi* appear to be very resistant. That *Stizolobium niveum* is highly resistant is shown by the fact that in a field test at Creedmoor, North Carolina, no demonstrable infection developed in any of the plants grown in wilt-infested soil. It is interesting to note that when young ragweed plants grown in the greenhouse were inoculated, they quickly succumbed to wilt, while numerous individuals grown out of doors when inoculated with the same strain showed no external symptoms of disease. Little external evidence of disease developed in rather mature plants of Croton but young plants were easily wilted. In the case of *Impatiens balsamina*, the foliage became slightly wilted, some distortion of the stems occurred and adventitious roots were formed. The discoloration of the vascular bundles of the stems showed through the cortical tissues as brown streaks. This species was found to wilt slowly when inoculation was effected by potting plants in infested soil. The discoloration of the vascular system is externally visible in wilted stems of *Eclipta alba* and the leaves become characteristically crisp and blackened.

The economic bearing of these additional weed and cultivated host plants for *Bact. solanacearum* upon the problem of wilt control is at once apparent when it is indicated that certain of these forms, namely; *Erigeron canadensis*, *Ambrosia artemisiifolia*, *Euphorbia nutans*, *Croton glandulosus*,

and *Solanum carolinense* are widespread in cultivated fields in the State. *Eclipta alba* is often found in ill-drained lands. *Datura tatula* and *Bidens bipinnata* are not uncommon weeds about farm buildings and lots. The Lycopersicons and *Martynia proboscidea* are locally rather common in gardens. The Tropæolums, Verbena, Impatiens, Browallia, Schizanthus, Salpiglossis and *Physalis alkekengi* are more or less commonly grown as ornamental plants. The results with velvet beans (*Stizolobium niveum*) which is related to Mucuna mentioned by Honing (6) are significant since this crop is becoming of considerable importance in the South. At least, it cannot be recommended that velvet beans be grown in a rotation system in soils infested with *Bact. solanacearum*.

SUMMARY

1. A wilt of tobacco caused by *Bact. solanacearum* has been observed in North Carolina in eleven counties and a tomato wilt caused by the same organism has been noted in thirty-nine counties.

2. Previous cultural studies on the identity of *Bact. solanacearum* from various hosts are confirmed since the variations which appeared in the strains from tobacco, potato, tomato, eggplant, peanut, ragweed and *Eclipta alba* were no greater than in strains all of which came from the same host.

3. A new family of phanerogams, Balsaminaceæ, has been added to the number previously reported to contain host species of *Bact. solanacearum*. Members of nine families are now known to be subject to attack by this organism. Thirteen additional species of plants, classified as follows showed well-defined wilting or serious injury:

Leguminosæ	*Stizolobium niveum*
Tropæolaceæ	*Tropæolum lobbianum*, *T. peregrinum*
Euphorbiaceæ	*Croton glandulosus* var. *septentrionalis*
Balsaminaceæ	*Impatiens balsamina*
Verbenaceæ	*Verbena erinoides*
Solanaceæ	*Lycopersicon cerasiforme*, *L. pyriforme*, *Browallia demissa*, *Physalis alkekengi*, *Schizanthus pinnatus*, *Salpiglossis sinuata*
Pedaliaceæ	*Martynia proboscidea*.

No outward signs of disease developed in the case of six other species in which the organism multiplied rapidly within the vascular portions. Five of these species are previously unreported, namely:

Euphorbiaceæ	*Euphorbia nutans*
Solanaceæ	*Solanum carolinense*
Balsaminaceæ	*Impatiens sultani*
Compositæ	*Bidens bipinnata*, *Erigeron canadensis*.

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SPARASSIS RADICATA, AN UNDESCRIBED FUNGUS ON THE ROOTS OF CONIFERS

JAMES R. WEIR

WITH FIVE FIGURES IN THE TEXT

In August, 1912, the writer collected several specimens of a species of Sparassis growing on the roots of various conifers in the Priest River Valley, Idaho. Lloyd, to whom specimens were sent, pronounced it an undescribed species. Cotton of the Pathological Laboratory at Kew who was advised by Lloyd of the writer's specimens stated that the plant was unknown to him. Since collecting the first specimens, the writer has studied the plant in several regions of the Northwest and finds that of the many peculiarities of the species the most surprising discovery is its evident parasitism on the roots of conifers. Although this fact was noted in 1912, it was not until the plant was carefully studied in its relation to its several hosts that this phase in its life history could be satisfactorily determined.

DESCRIPTION OF THE FUNGUS

Since the fungus does not agree with any known member of the genus, it is described as new.

Sparassis radicata n. sp.

Fruiting structure large, 12 to 22 cm. broad, 10 to 16 cm. high, dilated above, compact, fleshy, tough, whitish, creamy yellow with age, branched; branches numerous, horizontal or vertical, anastomosing, sometimes forming labyrinth-like cavities, more often compactly arranged, very thin, fan-shaped with wavy, sometimes deeply lobed margins, occasionally striated, amphigenous or unilateral, depending on the position of the branch, stalk, sclerotioid, tuberculate, firm, solid, sometimes branched, 20–30 cm. long, 5–8 cm. broad; spores, (50) range 2.8–4.0 × 2.8–5.5 μ, standard size 3.9–5.1 μ, ovoid, hyaline.

Type locality. Priest River, Idaho.

Habitat. Living roots in coniferous trees.

Range. Oregon, Idaho, Washington, Montana, and British Columbia.

Type material. In the Office of Investigations in Forest Pathology, Bureau of Plant Industry, Missoula, Mont.

GENERAL MORPHOLOGY AND TAXONOMY OF THE GENUS SPARASSIS

The genus Sparassis was established by Fries[1] and placed in the Clavariaceæ because of its frondose habit, fleshy consistency, and the belief that the spores were produced on all surfaces of the sporophores. It has recently been shown by Cotton[2] that the hymenium of Sparassis is not amphigenous but that the flattened branches with the exception of those in the center of the sporophore, are unilateral. On the basis of the flattened sporophore and the inferior hymenium Cotton suggests that Sparassis should be removed from the Clavariaceæ and placed in the Thelephoraceæ. He points out that in the Merisma section of the genus Thelephora are species with upright, partly unilateral sporophores either terrestrial or growing on wood which in many respects have the characters of Sparassis. In points of smoothness of the hymenium he further suggests that Sparassis is allied to Stereum but since the relationship to Stereum is not very close sees no reason why the genus Sparassis should not be transferred to the Thelephoraceæ without reference to any particular genus. Sparassis would then be distinguished as a genus of the Thelephoraceæ having fleshy, flattened, horizontal or vertical anastomosing branches with unilateral structures. The same view is entertained by Lloyd[3] who thinks the definition as laid down by Fries "fertile on both sides" should be corrected. Whether or not this view should be adopted in view of the fact that there is considerable irregularity in the formation of a unilateral sporophore is doubtful. In young sporophores of *Sparassis crispa* (Wulf.) Fr. examined by the writer, also of *Sparassis radicata*, the hymenium is by no means confined to the lowermost portion of the flattened branches but is found more or less uniformly over all free surfaces. This is particularly true, as Cotton points out, for those branches in the center of the sporophore but with a more pronounced unilateral structure toward the periphery. The hymenium of *Sparassis radicata* is formed very rapidly on the reverse side of the peripheral lobes when changed from their origninal position. A few specimens with unusually vertical lobes showed an amphigenous hymenium throughout making it seem probable that the lobes only become unilateral when they develop in a position allowing the influence of gravity to be more active on one side than another. There are, however, very few unilateral fungi, if any, that, under proper conditions of growth, will not when reversed develop the hymenium on the upper side.

[1] Fries. Systema mycol., I, p. 462.

[2] Cotton, A. D. On the structure and systematic position of Sparassis. Trans. British Myc. Soc. **1911**: 336-339.

[3] Lloyd, C. G. Letter No. 61, note 400.

Both Quélet[4] and Patouillard[5] noted the affinities of Sparassis with c tain groups in the Thelephoraceæ but apparently without definite knov edge of the hymenial development in the genus. Somewhat later Mai separated Sparassis from the Clavariaceæ making it the type of a spec family, the Sparassideæ. Although Maire's classification was adopted Lotsy,[7] critical work on the development of the hymenium, permanen

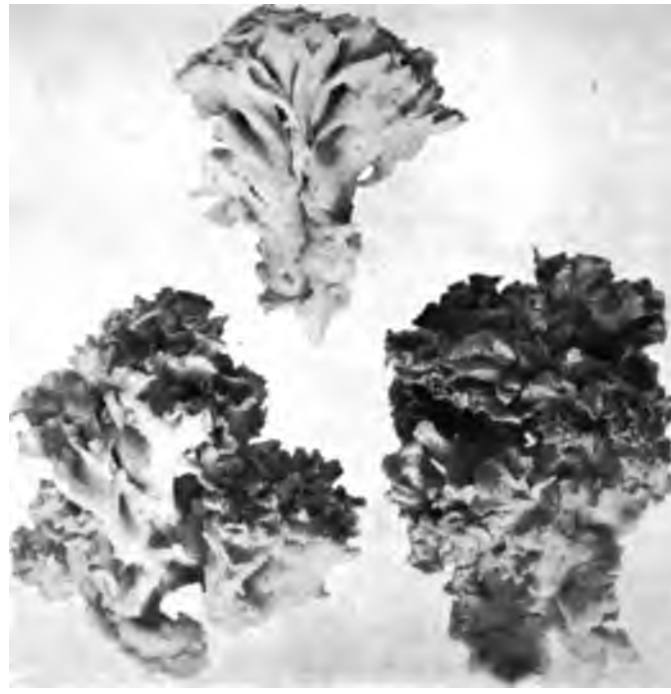

FIG. 1. SPARASSIS RADICATA

Small fruiting structures from a large sclerotioid stalk attached to the roots *Pinus montícola*

of the unilateral structure of the branches under various factors of grow and so forth is very much needed before the change should be accepte

Lloyd has made the suggestion to the writer that the genus Sparas falls naturally into two sections: first *S. crispa*, which is more of a flesl

[4] Quélet. Flora mycologique de la France. Paris. 1888.

[5] Patouillard. Les Hyménomycètes d'Europe. Paris. 1887.

[6] Maire. Recherches cytologiques et taxonomiques sur les Basidomycètes. A nexé au Bull. Soc. myc. France. **18**: -. 1902.

[7] Lotsy. Vortrage uber botanische Stammes geschichte. I. Jena. 1905.

nature especially near the base of the branches and is very closely related to Clavaria, and a second section consisting of species with thin lobes such as *S. spathulata* in the United States, *S. laminosa* of Europe, and *S. radicata* which has thinner lobes than either of the former. This seems to be a very logical arrangement. He further suggests[8] that *Sparassis laminosa* and *S. spathulata* are probably indentical. Their sporophores are certainly very similar.

THE ROOT STALK OF SPARASSIS RADICATA

Sparassis radicata (fig. 1) differs chiefly from *Sparassis crispa* (Wulf.) Fr. (*S. ramosa* Schäff.) which is reputed common in the eastern United States and in Europe, *S. laminosa* Fr. of Europe, and *S. spathulata* Schw. (*Stereum spathulatum* Schw.) (*sparassis Herbstii* Pk.) of America in the thinness of its lobes and by its very pronounced perennial sclerotioid rootstalk from which the sporophore develops annually (fig. 2). Since the rootstalk is usually attached to the deeper lateral roots of its host, it is often of a surprising length especially if a thick deposit of forest litter has accumulated around the base of the tree. Specimens have been found 50 cm. in length but the average is from 20 to 31 cm. No record exists of such a rootstalk for any other species. *Sparassis crispa* has a rooting base but it is not known to be perennial. It is possible that this phase of development is common to the other two species but has been overlooked. Sometimes the underground stalk is divided into two secondary ones each supporting a sporophore (fig. 2). The spongy character of the upper portion of the rootstalk soon merges into a very hard, compact mass and at the point of attachment to the root has very much the appearance of true cellular structure with the component filaments arranged longitudinally. The periphery of the stalk at the surface of the ground is composed of hyphae very much modified into a hard encrusting layer and may sometimes have a resinous appearance. The mycelium at the base of the stalk usually cements the earth into a hard stone-like body often of large dimensions. The fungus has not been found growing in the soil unattached to woody material. It is doubtful if it ever does so occur. All specimens so far collected were found at the base of trees.

The structure of the rootstalk is not that of a true sclerotium although it functions as such, is permanent and produces new sporophores from year to year. The stubs of old sporophores are plainly evident on the old root stalk (fig. 3) and as high as ten have been found on a single specimen. It was expected that the rootstalk would have great power of regeneration. This was tested on July 3, 1915 by cutting off a half-grown

[8] Lloyd, C. G. Letter No. 44, note 51, 1913.

sporophore. By August 30 a fully mature sporophore of average size had developed from the cut surface. On the former date also, a large root-stalk was severed from the roots of a Douglas fir, the fruiting end cut squarely off and the stalk buried loosely in moist forest soil. After a slightly longer time than that required for regeneration from the one attached to its host, a small fertile sporophore appeared from the normally

FIG. 2 FIG. 3

FIG. 2. SCLEROTIOID STALK OF SPARASSIS RADICATA

Showing lateral fruiting branch and tuberculate surface.

FIG. 3. UPPER PORTION OF SCLEROTIOID STALK OF SPARASSIS RADICATA

The remains of the sporophore of the season, two of the previous season, and two branches which produced sporophores in former years are shown. The fruiting nature of the latter was evident before the specimen was washed and dried.

fruiting end, showing not only the evident polarity[9] of the rootstalk but that it is a reserve structure of considerable reproductive power.

The formation of sclerotioid bodies from which their fructifications are developed is common to a number of Polypores. Chief among these noted in western United States are *Polyporus berkeleyi*,[10] *P. umbellatus*,[11] *P. frondosus*, and *Lentinus* sp., parasitic on the roots of conifers and probably unnamed. The latter species has a true sclerotium.[12] Lloyd[13] lists the following species growing from sclerotia-like structures and separates them as a distinct group of the section Ovinus of Polyporus: *Polyporus tuberaster* (Japan, China, and Europe), *P. Goetzii* (Africa), *P. Sapurema* (Brazil), and *P. Mylittæ* (Australia). Three other species also with sclerotia but not included in this section are *P. basilapidiodes* (Australia), *P. sacer* (Africa), and *P. rhinocerotis* (Malay).

The formation of sclerotioid bodies seems to be common to the Clavariaceæ. Some of the large species of Clavaria are observed to spring from large globose masses which when sectioned exhibit a very compact structure and are known to last over for more than one year. This has been observed by the writer for *Clavaria aurea*, *C. amethystina* and *C. formosa*. The members of the interesting genus Typhula always, so far as observed, produce sclerotia from which the sporophore is produced. In view of the fact that the sclerotia-forming habit seems to be more or less common to the Clavariaceæ, together with the fleshy consistency of the sporophores, flattened or cylindrical anastomosing branches, large size of many species, amphigenous hymenium, constant in most genera, irregular in others, it seems that this family is very well defined. The removal of the genus Sparassis to the Thelephoraceæ, which possesses few or none of these characters, would be, it seems, an unnatural arrangement.

[9] Weir, James R. Untersuchungen über die Gattung Coprinus. Flora n.s. **103**: 301–305. 1911.

[10] Weir, James R. Some observations on Polyporus berkeleyi. Phytopath. **3**: 101–103, pl. 9. 1913.

Later observed by Lloyd, Letter No. 60, note 391, 1915; and Letter No. 59, note 306, 1915; and by Overholts, The Polyporaceæ of the Middle Western United States. Washington University Studies **3**: 23, pl. 2. 1915.

[11] Lloyd, C. G. Letter No. 58, note 277, 1915; and Overholts in The Polyporaceæ of the Middle Western United States. Washington University Studies. **3**: 24, pl. 2. 1915.

[12] Petch, T. The pseudo-sclerotia of Lentinus similis and L. infundibuliformis. Ann. Roy. Bot. Gard. Peradeniya **6**: 1–18, pl. 1. 1915.

[13] Lloyd, C. G. Synopsis of the Section Ovinus of Polyporus. 74–76. Oct. 1911. Cincinnati, Ohio.

THE DISEASE CAUSED BY SPARASSIS RADICATA

The observation that possibly some members of the genus Sparassis are parasitic on the roots of forest trees has been made by others. In a letter to the writer, dated January 7, 1916, Doctor Cotton writes: "*Sparassis crispa* has been found frequently, and from its intimate connection with the roots of Pinus and other conifers we are strongly inclined to suspect that it is parasitic."

Kirchmayr,[14] it appears, was the first to entertain the suspicion that Sparassis had symbiotic or parasitic tendencies. Working with *Sparassis crispa*, he found that the stalk of this species penetrated deep into the earth at the base of the tree (*Föhre*). Boring into the roots from which the fungus appeared to have sprung, he found that after passing through a zone of healthy wood the auger encountered diseased wood. This wood was of a brown color, gave out a strong odor of turpentine, and was very soft so that the auger readily pushed through it. Two trees when cut showed that the brown rot extended up into the heartwood of the trunk for a distance of two meters. The decayed wood could be rubbed into a fine powder and gave out an odor of turpentine. The decay resembled that produced by *Polyporus sulphureus*, the checks extending vertically and paralleling the annual rings. The checks were lined with a fine mycelial layer which was encrusted with granules of calcium oxalate. Large peices of the cubical checked wood could be removed from the hollow in the heartwood. The heartwood in the larger roots was also decayed, while the sapwood was infiltrated with pitch ("*verkient*"). The decayed wood largely dissolved in ammonia producing a thick brown liquid which on neutralization held a brown deposit in suspension.

The author was unable to demonstrate the relation of the mycelium of *Sparassis crispa* with that in the diseased wood. He calls attention to the fact that the shrinkage of the wood in the form of cubes with surfaces covered with a fine white mycelial layer, brown color, odor of turpentine, and ability to be rubbed into a fine powder are characteristic of the decay produced by *Polyporus schweinitzii*. In the writer's experience the rot of *Polyporus schweinitzii* may not always be accompanied by the production of sporophores until a long time after the wood is well advanced in decay. Since direct connection of the mycelium of the base of the sporophore with that of the decayed wood was not discernible, it seems quite probable that the investigator has made an incorrect diagnosis.

A careful examination by the writer of six trees, the roots of which bore the fructification of *Sparassis radicata* has not revealed, with one excep-

[14] Kirchmayr. Über den Parasitismus von Polyporus frondosus Fr. und Sparassis ramosa Schaff. Hedwigia **54**: 334-337. 1914.

tion, the presence of such decay as described by Kirchmayr. The exception was a case in which an old decayed sporophore of *Polyporus schweinitzii* was found buried in the litter at the base of the tree. The rot in a part of the root to which the Sparassis was attached showed the characteristic decay of *Polyporus schweinitzii* but could be easily distinguished from the decay produced by the former species. This strengthens the writer's assumption that the trees examined by Kirchmayr might possibly have been infected with *Polyporus schweinitzii* or *P. sulphureus*,

FIG. 4. FAN-SHAPED MYCELIUM OF SPARASSIS RADICATA IN THE BAST OF A ROOT OF DOUGLAS FIR

the rot of which is similar and is also mentioned by the author in connection with his description. It is possible that *Sparassis radicata* produces a different decay from that of *Sparassis crispa*. If this is true, it is at least an argument in favor of the former being specifically different from *Sparassis crispa*.

The great depth to which the rootstalk of *Sparassis radicata* penetrates the forest soil has made the investigation of the diseased roots difficult. When attached to the deepest lateral roots, a considerable excavation

was necessary before the point of attachment was revealed. In the case of two of the trees examined dynamite was used to expose the infected roots over a considerable area. This method was found serviceable since the thicker roots were broken up in such a manner as to expose the diseased wood and the mycelium beneath the bark to good advantage. In most cases the earth was carefully dug away from around the rootstalk leaving the entire fruiting body of the fungus standing free.

Upon removing the bark from any of the infected roots to which the fungus is attached, a yellowish white mycelium is exposed. This mycelium develops in the living bast and ramifies in all directions in fan-shaped layers (fig. 4). After the death of the root, small rhizmorphs develop from the border strands of the original mycelial layer. These

FIG. 5. TYPICAL DECAY OF HEARTWOOD CAUSED BY SPARASSIS RADICATA IN THE ROOT OF A DOUGLAS FIR

strands break up into mycelial filaments and penetrate the outer sapwood producing a brown or yellowish, laminated, carbonizing rot. The shrinkage of the diseased wood causes very small, narrow, elongated pits or shrunken areas to appear but they are not lined with mycelium. As is the case in some other rots, the initial stage of decay occurs principally in the region of the medullary rays which may become wholly disorganized before the surrounding tissues are affected. Very small brown rhizomorpha occasionally develop in the decayed wood either paralleling the tracheids or extending in zigzag lines. The early stage of decay is always preceded by a reddish color. A jet-black line sometimes marks the boundary of the decayed wood. The completely decayed root shrinks slightly away from the bark leaving a space which is eventually filled

with a thick, mycelial mat from which the rootstalk takes its origin. The greatest decay occurs at the point where the rootstalk is attached and is at first confined principally to the sapwood. Small roots originating from larger ones to which the rootstalk is attached are usually decayed throughout. The early decay of the heartwood in the larger roots is probably prevented by the large amount of pitch which they contain. Eventually the heartwood is invaded but is not broken down uniformly. Elongated pits filled with a white mycelium are formed in different parts of the wood, often anastomosing in such a manner as to leave long pieces of partially decayed or solid wood which may be very readily removed (fig. 5). Sunken areas on these pieces correspond to similar pits on pieces which have become wholly disorganized. These elongated pits are often bounded by a white mycelium arranged in the form of a network. The tissue in the heartwood is brittle but can not be rubbed into a fine powder as described by Kirchmayr for wood attacked by *Sparassis crispa*. The rot of the heartwood is always of a darker color than that of the sapwood. In Douglas fir it is brown; in spruce, of a more yellowish color. Away from the seat of first infection the mycelium may advance into the innermost heartwood causing the formation of a pitchy zone next to the sapwood. The diseased wood may be drawn out of such roots in strips leaving a hollow cylinder. The cambium and outer bast are always, however, permeated by the mycelium in the characteristic fan-shaped masses. The action of the mycelium in the resin ducts of the bast causes a flow of pitch which may cement the soil to the root in stone-like masses.

The fact that the fungus can maintain its activity in the cambium in roots deep in compact, mineral soil is very unusual. Some of the root fungi which attack primarily the heartwood may follow the roots to a considerable depth, and *Armillaria mellea* and *Fomes annosus* habitually attack the cambium to a considerable distance in the mineral soil, but in the experience of the writer no other species has developed this ability to as great an extent as *Sparassis radicata*. The decay is apparently confined to the roots proper, never having been traced beyond the surface of the soil. In case of an excessive accumulation of forest débris around the base of the tree the decay may extend higher up on the lateral roots than is ordinarily the case when this accumulation of materials does not occur.

Only two species of fungi are definitely known to parasitise the roots of coniferous trees in the temperate zone, viz, *Fomes annosus* and *Armillaria mellea*. *Rhizina inflata*[15] may possibly be grouped here but in northwestern United States seems to be confined principally to seedlings.

[15] Weir, James R. Observations on Rhizina inflata. Jour. Agr. Research 4: 93-95. 1915.

There are a number of fungi which attack the roots of forest trees, are not strongly parasitic and do not cause a rapid browning of the foliage and rapid death. Their action is confined mainly to the heartwood of the roots and the base of the trunk. The most common of these is *Polyporus schweinitzii*. Other species which are either wholly confined to the roots and bases of trees or extend into the roots from infection through wounds on the trunk are *Trametes Pini*, *Echinodontium tinctorium*, *Polyporus sulphureus*, *Poria weirii*, and so forth. In the light of the present status of the study it can not be stated just how rapidly *Sparassis radicata* causes the death of its host. It has not been found on reproduction or young trees. The plant is not abundant but sufficient data have been assembled to show that it may be placed in the same group with *Armillaria mellea* and *Fomes annosus*.

To date only four trees, two Douglas firs (*Pseudotsuga taxifolia*), one white pine (*Pinus monticola*), and one spruce (*Picea engelmanni*) have been found to have succumbed to the action of the fungus. The conclusion that the death of these trees was caused by *Sparassis radicata* was arrived at because of the absence of any other fungus or factor which has heretofore been accredited as causing the death of trees. Several unhealthy trees with the fungus on their roots have been studied, but the common root fungi were present making a correct diagnosis impossible. The fungus has in every case, however, been found to cause the death of the living parts in the roots to which it was attached.

HOSTS AND DISTRIBUTION OF THE FUNGUS

Sparassis radicata is very widely distributed in the Northwest, having been found by the writer in British Columbia, Washington, Oregon, Idaho, and Montana. *Sparassis crispa* as reported from California is very probably based on this species.

The fungus has been found attacking the roots of the following conifers: *Pseudotsuga taxifolia*, *Picea engelmanni*, *Pinus monticola*, and *Larix occidentalis*. Its occurrence on the roots of broad-leaf species has not been noted by the writer. Kirchmayr cites instances of the occurrence of *Sparassis crispa* on oak and beech and other broad-leaf species.

SUMMARY

The large species of Sparassis in the western United States is found to differ in a number of details from *Sparassis laminosa*, *S. crispa*, and *S. spathulata*, and is described as new under the name *Sparassis radicata*.

The fungus is chiefly distinguished by its thin lobes and an unusu-

ally large perennial rootstalk which is of the nature of a sclerotium and from which new sporophores are developed from year to year.

The most important feature in the life history of the species is its parasitism on the roots of conifers. The mycelium attacks the bast of the roots and later the wood, producing a yellow or brown, carbonizing rot.

OFFICE OF INVESTAGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
MISSOULA, MONTANA

SOME CHANGES PRODUCED IN STRAWBERRY FRUITS BY RHIZOPUS NIGRICANS

NEIL E. STEVENS AND LON A. HAWKINS[1]

INTRODUCTION

The rot caused by *Rhizopus nigricans* Ehrenb. in strawberry fruits (*Fragaria* sp.) and potato tubers (*Solanum tuberosum*) has been studied by the writers. In both these cases as well as in others[2] the rot caused by this fungus is characterized by the rapid softening of the affected tissues accompanied by the loss of a large amount of juice. The morphological changes produced in the strawberry by *Rhizopus nigricans* have already been investigated. It was to obtain some information on the bio-chemical changes brought about in the strawberry fruit by this fungus that the present study was undertaken. In this work the effect of *Rhizopus nigricans* on the sugar, acid, pentosan, and crude fat content and the percentage of dry matter of the strawberry was studied.

METHODS

The berries used in this study were all of the variety Missionary, grown at Vienna, Virginia, and picked between May 20 and June 5, 1916. The methods for disinfecting and sampling used successfully by one of the writers in studies of peach (3) and potato (5) rots were found inapplicable to this work. The outer layers of cells of the strawberry were so injured by antiseptics, such as mercuric chlorid and alcohol, as to render results of doubtful value, especially as it had already been demonstrated that under normal moisture conditions the mycelium of the fungus grows chiefly in the outer cell layers (8). The texture of the strawberry, of course, prevents portions of the same berry being used for inoculation and control. The error due to variations in individual fruits may be somewhat greater than where portions of the same fruits can be compared.

[1] In the experiments described in this paper the cultural work was done by Stevens. The junior author is responsible for the chemical work. The writers are indebted to Mr A. A. Riley of the office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations for assistance in the chemical work.

[2] The literature referring to the effect of *Rhizopus nigricans* on various fruits has been briefly reviewed in another paper soon to be published. Stevens, Neil E. and Wilcox, R. B., Rhizopus rot of strawberries in transit. U. S. D. A. Bul. 531.

After some preliminary experiments the following method was found satisfactory and was followed throughout the work: Berries as nearly uniform in size as possible were picked when nearly ripe, i.e., when about half of the berry showed a bright red color. They were picked early in the morning while still cool and covered with dew. The calyxes were removed and the fruit washed several times in sterilized, distilled water. They were then placed in wide-mouthed flasks which had been plugged with cotton and sterilized. Three or four berries were usually placed in each flask. The berries were inoculated with spores and mycelium from pure culture, a strain of *Rhizopus nigricans* isolated from strawberries shipped from Florida during February, 1916, being used for inoculation.

The method usually followed in preparing the samples of fruit for analysis was to grind the berries in a mortar and then wash the pulp quantitatively into the proper container. The flasks with the berries in them were weighed immediately before the fruit was prepared for analysis and the washed and dried flasks were weighed again after the berries and juice had been removed. The wet weight of the fruit could thus be calculated. All determinations were related to wet weight of the sound or rotten fruit. The methods for the determination of the sugars, pentosans, and dry matter were similar to those followed in the studies of peach brown-rot (3) and the rots of potato (6).

The acid content was determined by grinding a sample of fruit, usually about 20 grams, in a mortar, then allowing it to stand three days in a flask with 150 cc. water to which a little toluol had been added. The acid was titrated with n/10 sodium hydroxid in this flask using litmus solution as an indicator. The end-point in these titrations was not as exact as might be desired because the pigments of the strawberry which were present in the solution made it impossible to detect slight changes in color. However, the determinations are all comparative and the differences in acid content in the sound and rotten berries are large. The crude fat determinations were made by extracting the dried and ground samples of fruit with water-free ether, which was then evaporated and the residue dried and weighed.[3] A number of samples of freshly picked sound berries were analyzed to obtain some idea of the variation in the content of the compounds determined between the individual samples. The results of these analyses are shown in table 1.

From table 1 it may be seen that there is some variation in the content of the compounds determined, especially the acids and sugars. The

[3] 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. Reprinted, 1912.

TABLE 1

Showing the content of sugars, pentosans, acids, crude fats, and dry matter in freshly picked strawberries. Three separate samples used in the determination of each substance

PENTOSANS AS PERCENTAGE WET WEIGHT	ACID AS CC. NORMAL ACID PER 100 GRAMS WET WEIGHT	REDUCING SUGARS AS PERCENTAGE WET WEIGHT	SUCROSE AS PERCENTAGE WET WEIGHT	CRUDE FATS AS PERCENTAGE WET WEIGHT	DRY MATTER AS PERCENTAGE WET WEIGHT
0.57	2.59	2.89	1.37	0.27	7.97
0.59	2.10	3.67	1.69	0.29	8.17
0.56	2.15	4.03	1.65	0.34	8.15

variation in pentosan content is slight. There is a considerable amount of sugar and the acid content is rather high. The berries of course were not ripe when picked and analyzed but were at about the stage of maturity at which they are harvested in some sections of the South where this variety is grown commercially. A comparison of the results of these determinations and the data obtained from the analysis of the sound strawberries which were analyzed three, seven, or fourteen days after harvesting gives some data on the changes which take place in picked strawberries during ripening.

In table 2 is shown the pentosan, acid, sugar, and crude fat content and the percentage of total dry matter in the sound and rotted samples of fruit at different times after harvesting. The analyses are also of some interest in showing the amount of the various substances in the strawberry fruit. Considerable work has, of course, already been done on this subject by various investigators. A review of much of this work is given by Wehmer (9, p. 284–285). In table 2 the results given are averages of at least three determinations of the various compounds on as many separate samples.

TABLE 2

Comparative pentosan, acid, sugar, and crude fat content and the amount of total dry matter in sound and rotten strawberries

NUMBER OF DAYS AFTER INOCULATION	PENTOSANS AS PERCENTAGE WET WEIGHT		ACID CONTENT AS CC NORMAL ACID, PER 100 GMS. OF FRUIT		REDUCING SUGARS AS PERCENTAGE WET WEIGHT		SUCROSE AS PERCENTAGE WET WEIGHT		CRUDE FAT CONTENT AS PERCENTAGE WET WEIGHT		DRY MATTER IN STRAWBERRY AS PERCENTAGE WET WEIGHT	
	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit	Sound fruit	Rotted fruit
3	0 51	0 51	1 98	1 89	1 95	1 35	1 89	0 66				
7			0 76	1 49	0 44	0 13	0 20	0	0 36	0 40	7 19	6 19
14	0 38	0 32	0 73	1 55					0 34	0 31	6 63	5 96

From the results shown in table 2 it seems that the pentosan content is no lower in the rotten fruit three days after inoculation than in the corresponding sound samples. There is, however, a decrease in the pentosans as calculated on a wet-weight basis after the fungus has acted fourteen days. It seems probable then that the fungus utilized a portion of the pentosans. It is interesting to note that there is a somewhat similar decrease in the percentage of dry weight in the inoculated berries in two weeks so that if the pentosans were calculated on the basis of dry weight at the time of analysis the percentage of the furfurol yielding substances in the sound and rotten fruit would be approximately the same.

The effect of the fungus upon the acids seems to be to reduce the acids slightly, as the acid content after the first three days is somewhat lower in the rotted samples than in the corresponding sound ones. The acid content of the sound berries decreased rapidly until at the end of seven days it was only about half that of the rotted berries, and a similar ratio is evident seven days later. From a comparison of the acid content of the sound berries in table 2 with that of the freshly picked berries shown in table 1 it is evident that there is a gradual decrease in the acidity the longer the berries are allowed to stand and that this decrease is much more rapid in the first week. That this decrease was not due to a neutralization of the acid by ammonia either in the sound or rotten fruit was shown by the negative results obtained from several series of ammonia determinations by Folin's method. The acid is apparently used up by the berry in its metabolism, probably in respiration. That the decrease in acidity in the sound fruit was greater than that in the rotted berries seems to indicate that the mechanism for the utilization of this acid is destroyed or its action inhibited by the fungus. The fungus apparently uses little of the acid.

The sugar content of the rotted berries is always lower than that of the sound fruit of the same series of samples. A comparison of the percentage of sugars in the sound fruit, as given in tables 1 and 2, shows that the sugar content rapidly decreases after the strawberry is harvested. The sugars as well as the acids are apparently used by the strawberries in respiration or in other metabolic processes. The much more rapid decrease in sugar content of the inoculated fruit is evidence that the fungus uses the sugars.

The percentage of ether soluble material in the sound and rotted berries, considered in the tables as crude fats, does not undergo any decided decrease when the berry is rotted.

The percentage of dry matter in the rotted berries is less than in the sound fruit.

As has been mentioned above, this strawberry rot is characterized by

a rapid softening of the tissue, the loss of water, and apparently a general collapse of the berry. It was considered of interest in this connection to determine the amount of sugar and acid present in the juice which escapes from the inoculated berries as compared to that in the juice of healthy berries picked at the same time and maintained under the same conditions of moisture and temperature. For this experiment the two samples of berries were picked, washed in sterilized water, and placed in liter flasks. The berries in the one flask were inoculated with Rhizopus in the usual way, while those in the other flask were maintained as controls. The flasks were filled to the same height and were allowed to stand in the laboratory for three days. The juice was then poured off the inoculated fruit and the berries of the control sample were frozen with carbon dioxid, and the juice expressed with a fruit press. The sugar and the acid content of the samples of juice from both lots of berries were determined according to the usual method. The results are shown in table 3.

TABLE 3

Sugar and acid content of juice from sound and rotted strawberries

SUGAR (PERCENTAGE)				ACIDITY IN TERMS OF NORMAL ACID	
Juice from sound fruit		Juice from rotted fruit		Juice from sound fruit	Juice from rotted fruit
Reducing sugar	Sucrose	Reducing sugar	Sucrose		
2.66	2.98	0.62	0.14	0.191	0.208

	JUICE FROM SOUND FRUIT	JUICE FROM ROTTED FRUIT
Δ	0.694	1.037
Diffusion tension in atmospheres	8.37	12.50

From table 3 it is apparent that the acidity of the juice from the diseased berries is slightly higher than that of the juice from the sound ones, while the sugar content is considerably lower. In table 2 it is shown that the sugar content of the diseased fruit three days after inoculation was decreased considerably below that of the control samples. This may, of course, account for part of the difference. However, the sugar content of the inoculated berries after three days is a little over half that of the sound fruit, while the sugar content of the juice that leaks out of the rotted fruit is about one-eighth that of the juice expressed from sound berries. The utilization of the sugar by the fungus, then, can hardly account entirely for the lower sugar content of the juice from the inoculated berries and much of the sugar must still remain in the infected fruit.

Other materials than sugars and acids are, of course, present in the strawberry juice. In order to obtain some idea of the amount of substance in this watery extract, freezing point determinations were made on the two samples of juice. These determinations were made with a Beckmann freezing point apparatus in the usual way. The depression of the freezing point (Δ) of these juices below that of distilled water and the calculated diffusion tension (7, p. 30–31) or osmotic pressure of which these juices are capable are shown below:

The freezing point of the juice from the rotted berries is considerably lower than that of the juice from the sound fruit. As calculated by this method the solution from the rotted berries has, obviously, a higher diffusion tension than that from the sound fruit. The juice from the rotted berries then is a more concentrated solution of some substance or substances than is the juice from the sound fruit. From these experiments with the juice from the sound and rotted fruit it is evident that the juice which escapes from the rotted berries contains at least a part of the soluble matter that is present in the cell sap of the berry before it is attacked by the fungus.

DISCUSSION

The effect of the fungus upon the various constituents of the strawberry as shown in the foregoing pages is much the same as has been shown for other fungi and other host plants in similar studies. Most fungi apparently utilize the sugars in their hosts when growing parasitically. This has been shown by one of the writers in the case of the brown-rot disease of the peach (3) and some of the Fusarium rots of potatoes (6).

That the fungi sometimes lower the pentosan content of their host when living parasitically has also been shown (4, 6). *Rhizopus nigricans* apparently does not utilize the acids of the strawberry to any extent. Some fungi are apparently able to use the acids in their host plant while others are not, probably depending on the ability of the fungus to assimilate the specific organic acids that are present in the host. Behrens (1, p. 700–706) has shown that the acids in apples can be used by fungi. Sclerotinia, however, apparently had little effect on the acids in the peach fruit (3).

In considering these results it should be remembered that at the time of the first analysis, i.e., three days from the time of inoculation, leak had progressed to an advanced stage. That is, the berries were flattened and a large amount of juice had escaped. At this time, as shown by table 2, only relatively slight changes have taken place in the amounts of the various constituents for which analysis was made. There is apparently no difference in the pentosan content between the rotted berries and

sound berries of the same age, and the difference in the amount of acid present is very slight. Some reduction in the amount of sugar, both sucrose and reducing sugar, has of course occurred but as the sugar was probably chiefly contained in the cell sap this change offers no clue as to the cause of leaking.

The bio-chemical studies have, then, served to confirm the conclusion derived frum the histological study that the changes, detectable by the methods followed, which have taken place in the cells of the strawberry at the time leak occurs are relatively slight. The histological study showed that the cell walls of the strawberry are seldom pierced by the fungous hyphae and that the protoplasm of the cells is only slightly altered in appearance, the nuclei in particular retaining their normal appearance until the cells are crushed.

In accounting for the loss of juice which occurs in strawberries attacked by *Rhizopus nigricans* the only tenable hypothesis seems to be that the fungus so affects the protoplasm of the cells, perhaps by secreting some toxin, that it is no longer capable of functioning as a semi-permeable membrane. In this connection it is interesting to note that Gortener and Blakeslee (2) have recently demonstrated the presence of a substance in Rhizopus which is extremely toxic to rabbits. Whether the protoplasm of the strawberry is killed at once by the fungus or whether it is anesthetized and rendered permeable to the material dissolved in the cell sap is an open question. Further investigations on this subject are planned.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

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WITCHES-BROOMS ON HICKORY TREES

F. C. Stewart

With One Figure in the Text

In Ontario county, New York, trees of the shell-bark hickory, *Carya ovata*, occasionally bear witches-brooms apparently caused by the fungus *Microstroma juglandis* (Bereng.) Sacc. During winter, while the trees are bare, the "brooms" are readily detected at a considerable distance. They are typical witches-brooms consisting of compact clusters of short, upright branches. They are of all sizes up to about two-thirds of a meter in diameter in the bare state and, of course, considerably larger when in foliage. As many as thirty "brooms" of various sizes have been observed on a single large tree.

The leaves on the "brooms" are yellowish green above, and white and mealy with Microstroma spores on the under surface. Usually, they are smaller than normal and much curled. In mid-summer they blacken on the margins, then wither and fall prematurely. The fallen leaves are not replaced by new ones as happens with the cherry witches-brooms caused by *Exoascus cerasi*. The branch bearing the "broom" is, usually, considerably enlarged at the point of attachment of the "broom" and often dead beyond the point of attachment.

The constant occurrence of *Microstroma juglandis* on the leaves leads to the belief that this fungus is the cause of the "brooms." Almost every leaf on every "broom" shows the fungus over its entire under surface while the leaves on all other parts of the tree may be wholly free from Microstroma. The presence of the fungus becomes evident as soon as the leaves unfold in the spring. This condition of affairs is not rare. It has been observed during seven consecutive seasons on a large number of "brooms" on nine separate trees in three localities—Geneva, Canandaigua and Victor.

On the other hand, *Microstroma juglandis* has long been known as a parasite on the leaves of walnut and hickory and is widely distributed in Europe and America; yet its association with witches-brooms has not been previously recorded. In fact, the writer has been unable to find any published account of witches-brooms on hickory trees. The writer, himself, has occasionally observed *M. juglandis* on the leaves of hickory trees which bore no witches-brooms. At Geneva, in 1916, this was of com-

mon occurrence. In such cases the fungus appeared on the under surface of yellowish spots or small areas instead of covering the entire lower surface of the leaf. This fungus was morphologically indistinguishable from that occurring on the leaves of the "brooms."

In only a single instance has the writer had an opportunity to study a "broom" in its incipiency. This was a "broom" discovered on June 10, 1914. It consisted of a single new shoot growing vertically from a

FIG. 1. WITCHES-BROOMS ON A HICKORY TREE, CARYA OVATA

This tree bore seventeen "brooms" the largest of which was nearly a meter in diameter.

small enlargement about 13 cm. back of the tip of a branch 1 cm. in diameter. On the parent branch beyond the point of attachment of the "broom" the bark was stil green, but there were no leaves and the terminal bud was dead. Apparently, the infection had occurred in 1911. The "broom" arose from wood three years old.

The new shoot constituting the incipient "broom" bore seven leaves, three of which were free from fungus, while on each of the other four some

of the leaflets were attacked by Microstroma. On an adjacent branch there was a larger "broom" every leaf of which was covered with Microstroma, but none of the other leaves on the tree were affected. A later examination, made on July 2, revealed no change in the "broom" except that the margins of the affected leaflets had begun to blacken. Further observations were impossible owing to the accidental destruction of the young "broom."

NEW YORK AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK

A NEW LEAF-SPOT DISEASE OF CHERRIES

BERT A. RUDOLPH

WITH THREE FIGURES IN THE TEXT

In August, 1913, a conspicuous leaf-spot disease of sweet cherries was observed by the writer near San Jose, California, and specimens have been received from the principal cherry growing sections between Redlands, California, and Corvallis, Oregon. No mention of it has been found in any of the literature examined. Descriptions were sent to F. C. Stewart, Geneva, New York; Howard S. Reed, Blacksburg, Virginia; M. B. Waite, Washington, D. C., and Donald Reddick, Ithaca, New York, and all expressed doubt as to its occurrence in their localities. It is believed to be peculiar to the Pacific coast.

Dead, definite, circular spots from one to about 14 mm. in diameter appear on the leaves. The dead areas are a pronounced reddish brown or chestnut to mahogany color, and are sharply differentiated from the living tissue, extending from epidermis to epidermis and commonly marked with a delicate, concentric zonation consisting of narrow lines and darker bands. Several spots may coalesce to form one large one.

On the upper surface of the leaf may nearly always be found a minute, whitish gray pustule located in the center of the spot, and about which the zones are concentric. The pustules are uplifted bits of epidermal leaf tissue, and a minute insect of the family Chalcididae has regularly been found in them.

Within the spot there may be a definite, central, circular portion which is lighter than the remainder. This inner area sometimes reaches 6 mm. in diameter and is ochraceus to ferrugineus (Saccardo's Chromotaxia), or it may be entirely absent. The darker portion is castaneus to badius. The pustule, if present, is located in the center of the inner, lighter area, if such an area occurs. The under side of the spot presents a slightly different color from the upper. The inner lighter area is isabellinus, and the remaining portion lateritius, being lighter than the corresponding area on the upper surface.

The dead tissue remains intact within the leaf.

Sometimes the spots spread out in an irregular, somewhat indefinite, mosaic-like fashion (fig. 1).

The spots just described are produced by a fungus of the genus Alternaria which usually gains entrance to the leaf tissue through the injuries made by insects which produce the pustules. Cultures of the causal organism were obtained as follows: The leaves were first lightly sponged with alcohol. Bits of the spots were removed with a flamed scalpel and planted in plates of non-nutrient agar. From these plantings an Alternaria and

FIG. 1. SPOTS PRODUCED BY ALTERNARIA CITRI PIERCE VAR. CERASI ON CHERRY LEAVES

At the apex of one leaf is a mosaic-like spot produced by this organism. Insect pustules by which infection took place are seen in the center of the spots.

three other distinct fungi developed in more or less homogeneous colonies. Plantings were taken from these and placed in individual plates of non-nutrient agar and a homogeneous culture was obtained of each fungus. On October 16, 1913 these fungi were inoculated into young leaves of seedling cherries in the conservatory of the University of California.

Four leaves on one tree were inoculated, each with a specific fungus.

The leaves were first lightly sponged with alcohol. Bits of agar, bearing spores and mycelium, were taken with a flamed needle and placed on the surfaces of the leaves, and the needle was pushed through the bits of agar into the leaf tissue. The tree was then sprinkled with tap water and covered with a bell-glass. Within four days four large spots were produced on the leaf which had been inoculated with the Alternaria. The spots were more or less circular with diameters of about 7 mm. and were characterized by being of a wilted appearance, and of a greenish-brown color, and having three or four light-colored zones. The bell-

FIG. 2. BLACK HEART PRODUCED IN A NAVEL ORANGE BY INOCULATION WITH ALTERNARIA CITRI VAR. CERASI

glass was removed, and the killed areas grew slightly larger and lighter in color as they dried out. The inoculations with the three other fungi gave negative results. About twelve inoculations were made with each of them soon after on healthy cherry leaves, taking care to prick the tissue in each case, but the results were negative. Bits of the artificially produced spots were planted in nutrient agar, and a copious growth of but one fungus—the Alternaria-developed. A pure culture of this strain was then secured by the single-spore method and was used in all succeeding inoculations.

The details of subsequent inoculations are recorded in the writer's thesis, deposited in the library of the University of California. In all, over two hundred inoculations were made on the following varieties of cherries; Royal Ann, Richmond, Morello, Black Tartarian, seedling sweet cherry and California Wild Cherry (*Prunus ilicifolia* Walp.). During the winters of 1913 and 1914 experiments were confined to the leaves of seedlings. In the spring of 1914, however, large numbers of inoculations were made upon the various named varieties. The method of inoculation was the same as that described earlier. Checks were made on one side of the mid-rib and inoculations with a flamed needle on the opposite. Not more than ten of all the inoculations made in this manner were negative. In most of the inoculations no bell-glasses were used, and the spots developed quickly, although less rapidly than where covered. About twenty other inoculations were made in lots of two to five by laying bits of the fungus in agar on the leaves without puncturing or injuring them in any manner. All these were negative. The greater part of this work was done in a greenhouse without heat.

The fungus is particularly active when inoculated in the leaves of California Wild Cherry. About three dozen inoculations were made on this plant, and not more than five proved unsuccessful. Frequently artificially inoculated leaves were so badly affected as to be shed from the tree. This was especially common when the trees were kept under bell-glasses. The spots produced differ greatly in color from those on the leaves of sweet cherries. There is usually a circular, inner area in each spot which is avellaneus on the upper side and isabellinus on the lower. The remaining or outer portion of the spot is isabellinus, and the lower side latericius. At times the spots may be a deep brown color, especially when formed more slowly.

Microtome sections made of the freshly produced spots stained with congo red and methylene blue show best the action of the fungus. The parasite is intercellular. The chloroplasts of the cells lying just beyond the tips of the advancing mycelium are first affected and cannot be distinguished. The cells collapse and disintegrate rapidly as the fungous threads come in contact with them.

All inoculations in the bark and wood of normal cherry twigs were negative. Over two dozen inoculations were made on stems up to 2.5 cm. in diameter. The bark was first sponged with alcohol. Slant cuts were made with a flamed scalpel and the infectious material placed beneath the flap, or inoculations were made by puncturing the bark through the spore-bearing material. Both types of inoculations were either left exposed or wrapped with thoroughly boiled linen strips or bound in absorbent cotton. Checks were also made in the same way. The wounds healed normally, the plants apparently being unaffected by the fungus.

When inoculated in the leaves of other plants the results are often as pronounced as in the cherry leaves. The leaves were first sponged with alcohol and the inoculations and checks were made in the usual manner. The results obtained are shown in table 1.

TABLE 1

Results of inoculations of leaves of various hosts with a species of Alternaria from cherry leaves

HOST	NUMBER OF INOCULATIONS	RESULTS	OBSERVATIONS
Apple (Pyrus Malus L.)			
Winesap	20	All positive	Large reddish spots
Newtown Pippin	25	All positive	Large reddish spots
Box Elder (Acer negundo L.)	50	All positive	Large brown spots
Hungarian prune (Prunus domestica L.)	18	All positive	Small brown spots. Developed slowly
Wickson plum (Prunus triflora Roxb.), (P. Simonii Carr.) hybrid	26	All positive	Small brown spots. Developed slowly
Orange (Citrus nobilis Lour.)			
King Mandarin	20	Doubtful	Spots barely larger than on the checks
Loquat (Eriobotrya japonica Lindl.)	21	Negative	Spots no larger than on the checks
Potato (Solanum tuberosum L.)	30	Negative	Spots no larger than on the checks
Avocado (Persea gratissima Gaertn.)	32	All positive	Large reddish brown spots
Watermelon (Citrullus vulgaris Schrad.)	16	All positive	Black spots. Developed slowly
Peach (Prunus persica S. & Z.) Crawford peach	40	Positive	Irregular gray-brown spots

In general it was found that the fungus produced its optimum growth in the leaf tissue when the atmosphere was moist and warm and sunlight at a minimum. The mere shading of an infected leaf with a piece of paper was found to permit the production of larger spots in a shorter time than where the leaves were exposed to direct sunlight. The fungus is a typical wound parasite, all inoculations on uninjured leaves having failed. When a young leaf was inoculated before being fully developed a shot-hole effect sometimes resulted on its expansion.

The fungus grew vigorously on the common culture media, and its more important characteristics are as follows:

On non-nutrient agar. Growth rapid, mostly confined to the surface of the medium which is not discolored. A small amount of long, aerial, dry, silky, gray-white mycelium is produced. Spores thinly scattered over the surface of the medium.

On nutrient agar (containing meat extract, peptone and salt.) Growth vigorous, a copious aerial, downy mycelium is developed consisting of long, branched, silky, gray hyphae. Spores commonly produced in greatest numbers in concentric zones which are dark green at first becoming sooty black with age. The spores are olivaceus under the microscope. The agar is cleared of any cloudiness by the fungus as it develops.

On steamed rice. Growth vigorous. A snow-white, aerial, downy mycelium first develops which darkens to a dirty, greenish gray with age. A flesh-colored pellicle is produced upon the surface of the medium darkening with age to black. The rice grains gradually become colored a light yellow. Spores are produced close to the surface of the pellicle and beneath the longer aerial hyphae. They are pale olivaceus under the microscope.

On bread and prune juice (Duggar's Fungous Diseases of Plants, p. 24). Growth vigorous. A copious, downy, aerial mycelium is produced which is dull white at first becoming a dirty greenish gray with age. Patches of older parts of the aerial mycelium are often yellowish. A cream colored pellicle is formed on the surface of the medium becoming black with age. Spores develop close to the pellicle beneath the longer, aerial hyphae. They are dark olivaceus under the microscope. The medium becomes darker as the fungus develops upon it.

On steamed potato slants. Growth vigorous. A white, downy, aerial mycelium is first produced becoming a dirty greenish gray with age. A pellicle is formed upon the surface of the slant and may be flesh colored or greenish, becoming black with age. Finally the aerial mycelium usually collapses, and only a black, shining pellicle is observed. After growth has entirely ceased the plugs no longer react for starch with iodine but give a good test for reducing sugar with Fehling's solution.

On beet agar. Growth vigorous. An aerial, downy mycelium is first produced which is gray-white becoming greenish and finally black with age. Cultures have a sooty, granular surface punctuated with whitish hyphae in scanty tufts and occurring singly. The aerial mycelium may or may not be somewhat zonate. Spores are inclined to be smaller and decidedly darker than those found on other media, being olivaceus to fuligineus under the microscope.

On steamed cherry twigs. Growth vigorous. A copious, downy, aerial, white mycelium is first produced which becomes a dirty greenish gray to black with age, giving the cut surfaces a sooty appearance. The bark

is sparsely covered. Spores are produced in abundance close to the cut surfaces of the twigs. They are somewhat smaller than those on various other media and are dark olivaceus to fuligineus under the microscope.

On navel oranges (Sterilized by washing the surface with mercuric chloride solution). When inoculated in moist chambers at the navel end a black rot of the rag or pulp cells results which is identical with that produced by *Alternaria Citri* Pierce (fig. 2). At the point of inoculation an aerial mycelium develops which is pulvinate and gray-white at first, becoming a dirty greenish gray with age. The rind discolors becoming olivaceus in a gradually increasing area around the fungus colony. Several months after inoculation the aerial mycelium, having overrun the orange, bleaches out and ultimately becomes a beautiful pink. The whole fruit gradually settles down with a soft, moist rot.

On + 5 nutrient agar agar. This medium remains liquid due to the high acid content. The isolated colonies of aerial mycelium are whitish at first becoming sooty black with age and rounded or hemispherical. On titrating the medium three weeks after planting it was found to have been reduced to + 4. The color was changed from a light amber to a deep brown (fuligineus). This destruction of acid by the fungus was observed on various other media. The average of four titrations was always taken.

In general it was observed that the color, shape and size of the spores produced on various media may vary slightly, but the most important characteristics remain the same.

The fungus bears a striking similarity to *Alternaria Citri* Pierce and even a closer relationship to an Alternaria found on watermelon leaves. Pure cultures of the three fungi were obtained by the single-spore method. In drop cultures of +2 nutrient broth the fungi may be said to be identical morphologically. Possibly the spores of *A. Citri* are slightly rougher than those of the other two, but this difference was not found to be constant. The three fungi cannot be differentiated on nutrient and non-nutrient agar, and the rots produced by them in navel oranges are identical.

The cherry Alternaria cannot be distinguished from the watermelon Alternaria on +5 nutrient agar, but is distinguished from *A. Citri* on this medium. The latter produces colonies which are circular, white at first, becoming gray with age. They are also flat or depressed with crater-like centers which are darker in color (griseus-olivaceus).

The cherry Alternaria is distinguished from the other two Alternaria when inoculated on cherry leaves only by the size of the spots and the rapidity with which they are produced. The watermelon Alternaria produces the smallest spots, and they develop more slowly, but the difference is very slight. Over four dozen inoculations were made with *Citri* and the watermelon Alternaria.

The cherry Alternaria was distinguished from the other two by its action on watermelon leaves (var. Cuban Queen), being the least virulent of the three fungi. Sixteen inoculations each were made with the cherry Alternaria and *A. Citri* on separate plants. Twenty-four inoculations were made with the watermelon Alternaria on a third plant. The black spots produced were identical, but the watermelon Alternaria spread to the stem, killing the plant, while the cherry Alternaria confined itself to the leaves. *A. Citri* formed slightly larger spots than the cherry Alternaria but also confined itself to the leaves.

The cherry Alternaria may be further distinguished from the other two in the matter of spore germination. Fresh spores of the cherry Alter-

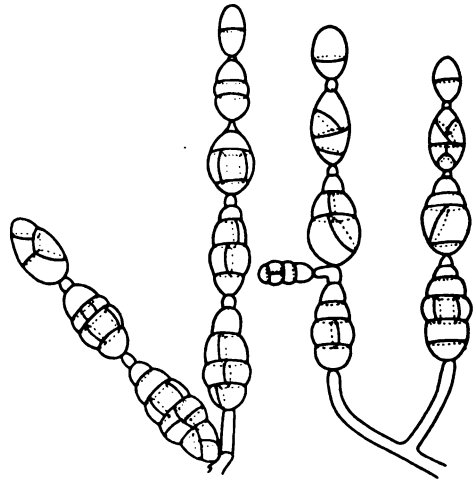

FIG. 3. SPOROPHORES AND SPORES OF ALTERNARIA CITRI VAR. CERASI

From drop culture of + 2 beef broth with peptone. × 500

naria will germinate in neutral, +1, and +2 broth in less than twenty-four hours, but in +7, and +8 broth germination rarely takes place in less than a week. It will grow and produce spores in +8 nutrient broth. Spores of the watermelon Alternaria will germinate in +8 broth and grow feebly, but the fungus has rarely been observed to produce spores in this medium. Spores of neither will germinate in +9 broth. *A. Citri* will grow and produce spores in +6 broth, but spores will not germinate in +7 broth. Spores of both the cherry and watermelon Alternarias germinate in +7 and +8 broth with the greatest difficulty, the germ-tubes developing in an abnormal manner and resemble the budding of yeast. Ordinarily the germ tubes are straight with but few septa, but in broths of high acidity, the opposite is the rule.

The cherry Alternaria cannot be distinguished from the others by germination tests in alkali solutions. All germinate readily in tap water containing 8 per cent normal alkali (sodium hydroxid), but in −10 tap water germination may not take place for a week. None of the three fungi will germinate in −11 tap water.

On steamed rice the watermelon Alternaria colors the rice grains a slightly lighter shade of yellow than does the cherry Alternaria, while *A. Citri* colors them all shades of yellow to latericious. In other respects the fungi are identical on this medium.

On +5 nutrient agar the growth and acid destruction by the cherry Alternaria was identical with that of the watermelon Alternaria. *A Citri* within the same time only reduced the acid content 0.5 per cent instead of 1 per cent.

The longevity of the cherry Alternaria is largely dependent upon the presence of moisture. However, spores over fourteen months old produced on steamed cherry twigs, which dried out soon after the planting of the fungus on them, were found to be viable, although germination rarely took place in less than a week in neutral or +1 broth.

The various experiments enumerated show the three fungi to be very closely related. The name of the watermelon Alternaria is not known, but it is not believed to be *A. cucurbitæ* Let. which is also parasitic on melon vines. The spores of *A. cucurbitæ* are described as longer and narrower—mostly 60 to 68 by 8 to 9 μ—while the spores of this particular Alternaria are only 10 to 47 by 6.8 to 15μ.

The cherry Alternaria is not believed to be the same as *Alternaria Cerasi* Potebnia found at Kharkov, Russia, the spores of that fungus being decidedly larger and produced in velvety patches on dry margins of leaves, according to Saccardo.

Believing this fungus to be hitherto undescribed, and being of the opinion that its close relationship to *A. Citri* Pierce entitles it to be classified as a variety of that species the writer suggests the name *Alternaria Citri* Pierce, variety *Cerasi* with the following technical description.

Alternaria Citri Pierce var. **Cerasi** nov. var.

Producing dead spots on leaves of sweet cherry. Spots 2 to 14 mm. in diameter, reddish brown or chestnut above and lighter below, often faintly zonate, sometimes with a distinct, lighter colored central area and usually starting from an insect injury, sometimes extending outward in an indefinite mosaic.

A wound parasite only, as shown by artificial infections, capable of producing spots on leaves of numerous plants. Distinguished from *A. Citri* Pierce with difficulty.

Mycelium, in leaves of sweet cherries, sub-epidermal, of slender, septate, hyaline hyphae, 1 to 3 μ in diameter. Aerial mycelium very rarely produced and then only in the presence of unusual humidity and optimum temperature, hyphae 3.4 to 4.8 μ wide, gray-white to pale olivaceus, long, silky, branched, septate; conidia, not observed in field, occasionally produced in presence of unusual humidity and optimum temperature—then close to the lower surface of the leaf, never on long, aerial hyphae, clavate fusiform or elliptical at maturity, 15.3 to 57.8 by 6.8 to 15.3 μ, muriform, translucent, olivaceus-brown, slightly verrucose or smooth, becoming constricted at the septa with age, transverse septa commonly parallel, 3.3 to 9.9 μ apart, with short, smooth, hyaline-subhyaline isthmus which is usually 3.4 by 3.4 μ; several catenulate, in cultures 2 to 7 in simple or branched series, somewhat variable in size, form and color, germination from any cell, produced abundantly on various culture media. Conidiophores, short, in cultures 1.7 to 153 μ by 1.7 to 6.8 μ, olivaceus-subhyaline, erect, in general narrower than the vegetative hyphae.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

BLISTER SPOT OF APPLES AND ITS RELATION TO A DISEASE OF APPLE BARK

DEAN H. ROSE[1]

WITH THREE FIGURES IN THE TEXT

In the present paper is described a disease of apples (fruits) which has been under investigation through two growing seasons. A brief report has already been published by the writer[2] but so far no other mention of it has been found in the literature. There is given also a description of a disease of apple bark which seems to be causally related to the fruit disease. The experimental proof is not yet complete but considerable evidence that the relation actually exists has been obtained and will be found summarized in the second part of the paper.

BLISTER SPOT OF APPLES

Occurrence and general appearance of the disease

The blister-spot disease was first noticed on July 6, 1915, on Norfolk Beauty (dwarf) as roughly circular or sometimes irregularly lobed shallow blisters, varying in color from light brown to black, and in size from 1 to 5 mm. in diameter (average about 2 mm.) by 0.2 mm. in depth. Search through the experiment station orchard at Mountain Grove, Missouri, then showed similar spots on Melon, Ishewold, and Hawley. In 1916 spots were first found about the middle of June on Blue Pearmain, Higginbotham, Yellow Transparent, Benoni, Melon, Rock Pippin, Lansingburg, Early Ripe, Victuals and Drink, Isham, Ishewold, Hawley, Norfolk Beauty, Red Astrachan, White Pippin (wrongly given as Yellow Newton in the report mentioned earlier),[2] Klondyke, Duling, and Jonathan. Allowing for the fact that such varieties as Yellow Transparent and Red Astrachan were gone when affected apples were discovered in 1915, this list shows the disease much more prevalent in the station orchard in 1916 than in 1915. Little is known of its distribution. Apples showing typi-

[1] The writer wishes to acknowledge his indebtedness to Mr. Harold Swartout and Miss Beatrice White, without whose careful and efficient help the work here reported could not have been accomplished in the time available.

[2] Rose, Dean H. Blister spot of apples (abstract). Phytopath. 6: 110. Feb. 1916.

cal spots, variety unknown, were received in May, 1916, from Marionville, Missouri, and what looked like an early stage of the disease was observed in 1915 on Ben Davis at West Plains, Missouri. No cultures were made from these apples.

Lesions occur at the lenticels and usually on the lower half of the apple. Benoni and Jonathan show spots only around the blossom end, while Higginbotham, Melon and others may be affected over any part of the surface except the upper one-fifth (fig. 1, *A*).

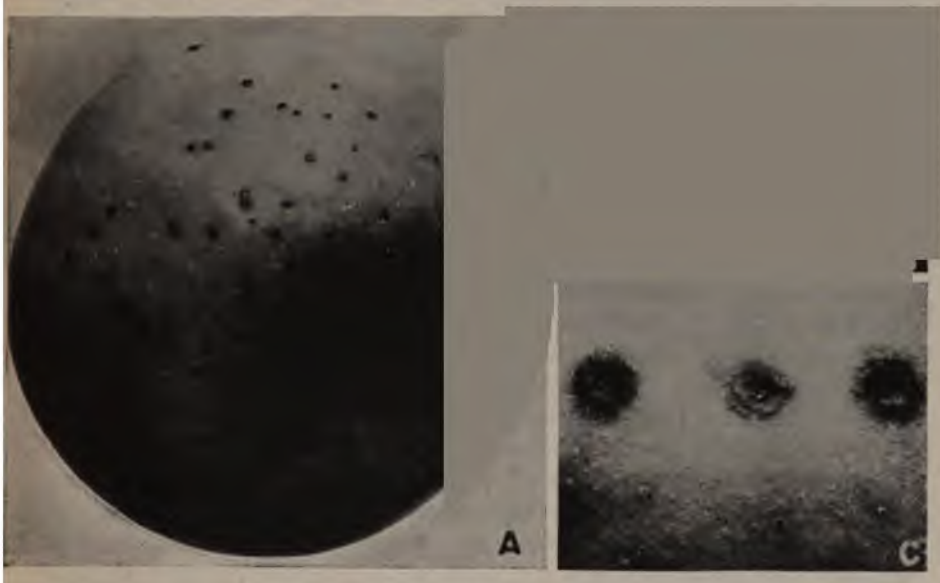

FIG. 1. BLISTER SPOT OF APPLES

A, Higginbotham apple showing natural infection. Spots roughly circular. Natural size.

B, Natural infection on Melon apple showing irregularly lobed appearance of blister spots. × 2.

C, White Pippin apple showing blister spots around inoculation punctures, three weeks after inoculation (August 27, 1916), with the blister-spot organism. × 4.

From a study of various stages on the same apple it was found that the first sign of the disease is a slight darkening around the lenticel, followed by the formation of a whitish to pale brown blister, 0.2 to 0.5 mm. in diameter. In later stages the epidermis over this blister becomes dark brown to black and dies. It may or may not crack loose from the healthy epidermis around it. If it does, the spot usually grows no larger; if it does not, the lesion may extend outward in various directions—Melon, Norfolk Beauty, Higginbotham, Klondyke—giving the irregularly lobed

appearance described above (fig. 1, *B*). On white or yellow apples such as Yellow Transparent the spots are surrounded by a narrow greenish ring; the same ring is found on Benoni and Duling, which are red when ripe, but the spots on Red Astrachan show a red ring.

The disease is not a serious one in the way that bitter rot is serious. Nevertheless, susceptible varieties often have the whole crop so seriously blemished as to be unfit for packing in any but the lower commercial grades. Fortunately such severe injury seems to be confined to varieties of minor commercial importance.

Cause of the disease

The disease is caused by an organism belonging to the genus Pseudomonas. It does not seem to have been described previously and the following name is proposed and a description appended:

Pseudomonas papulans n. sp.

Morphological characteristics. The organism is a rod, motile by one to six polar flagella. Flagella occur at both poles and were demonstrated by means of Loeffler's flagella stain, using stains on *Pseudomonas fluorescens* as check. (*Ps. fluorescens* furnished by the American Museum of Natural History, New York). Stained from twenty-four-hours-old agar cultures the organism shows as a short rod with rounded ends, measuring 0.9 to 2.3 μ long, by about 0.6 μ in diameter. It occurs singly and very often in pairs. No spores or capsules have been demonstrated. It stains readily with carbol fuchsin, gentian violet, and methylene blue. It is not acid fast and it does not stain by Gram.

Cultural characteristics. The following account is based on a study of 25 strains of the organism isolated from (1) naturally infected spots, (2) artificial infections, and (3) infections produced with the reisolated organism. Cultures were compared with *B. coli* and *B. amylovorus* (furnished by the American Museum of Natural History, New York).

The organism does not form gas from peptonized bouillon containing dextrose, saccharose, maltose, lactose, glycerin, or mannit, and it does not cloud the closed end of the fermentation tube in any case. Growth stops short in the neck of the tube, indicating an obligate aerobe. The organism clouds beef bouillon +10, slightly in twenty-four hours, and moderately in forty-eight hours. It liquefies gelatin slowly at 20°C., liquefaction not being complete in test-tube cultures until after twelve to fourteen weeks. Some strains form a soft coagulum in plain milk, some no coagulum at all, but all of them clear it in about twenty days. The organism blues litmus milk throughout during the first six days,

with the formation usually of a soft coagulum, and then gradually decolorizes it from above, with the production of a dark blue[3] color throughout after sixteen to twenty days. On plain agar it produces a filiform, slightly convex, whitish growth. On potato cylinders it produces, after forty-eight hours, a whitish, filiform, irregularly spreading growth, which after seven days shows a slight browning, accompanied by a slight darkening of the medium. The optimum temperature seems to lie between 25° to 28°C., though fairly good growth takes place at 20°C. It does not grow at 37°C. The thermal death-point has not been determined. On dextrose agar and glycerin agar the organism produces a light green fluorescence as also in Uschinsky's and asparagin solutions, but it does not grow in Cohn's solution. A test with six strains showed that the organism grows in bouillon over chloroform and tolerates hydrochloric acid up to +15 on Fuller's scale, and sodium hydroxide to −5. The optimum reaction for growth seems to be about +10.

The organism is sensitive to sunlight. Petri dishes one-half covered with black paper and exposed to sunlight, on August 3, on a bag of crushed ice showed, for six strains, an average of 98 per cent killed after an exposure of ten minutes. Six strains inoculated into test-tubes containing different amounts of sodium chloride showed growth in all up to and including 4 per cent. Using the method described by Edson and Carpenter[4] it was found that the organism produces alkali in plain milk during the first ten days, then increasing amounts of acid up to forty days. On peptonized bouillon containing two per cent of various sugars and alcohols it produces acid from dextrose and saccharose, alkali from lactose, and maltose, and neither acid nor alkali from glycerin or mannit.

Quick tests for differential purposes; bluing of litmus milk followed by decolorization from bottom upward accompanied by slow digestion and the formation usually of a soft coagulum; fluorescence and luxuriant rugose growth on glycerin agar; fluorescence on neutral gelatine but none on gelatine +10.

Isolation of the organism. The organism was easily isolated from affected apples by the method of poured agar plates. Spots were merely given a good washing with sterilized, distilled water, sometimes preceded by a brief rubbing with a finger dipped in alcohol. The diseased material was then scraped off with a sterilized scalpel and dropped directly into melted agar. The colonies appeared in from thirty-six to forty-eight hours, usually in pure culture. They were thin, smooth, circular, glistening,

[3] Saccardo, P. A. Chromotaxia seu Nomenclator Colorum. 1–22. 2 pl. Pata vii, 1894.

[4] Edson, H. A. and Carpenter, C. W. Micro-organisms of maple sap. Vermont Agr. Exp. Sta. Bul. **167**: 321–610. 1912.

whitish by reflected light, bluish by transmitted light, and 0.1 to 1.0 mm. in diameter. Not all the affected varieties were used in this work but no difficulty was experienced in obtaining pure cultures from those that were used.

Inoculation. Using sub-cultures from single colonies, the disease has been reproduced on six varieties of apples. In this work 123 apples were used, 60 of which were checks. All of them were treated on the trees and were bagged after treatment. The incubation period averaged about fourteen days, though some apples failed to show signs of the disease until the end of eighteen to twenty-five days. Some strains of the organism were infectious on all the varieties tested, others on only one or two. Further work is necessary to clear up this situation.

Inoculations were made in three different ways, using twenty-four to forty-eight-hours-old bouillon cultures:

1. By spraying uninjured apples with the bouillon culture. No infection resulted.

2. By spraying apples which had first been pricked with a flamed needle. Seventy-two per cent infection resulted on Yellow Transparent, Jonathan, Melon, Hawley, and White Pippin. No signs of infection appeared on Benoni.

3. By hypodermic injection just under the epidermis. Eighty per cent infection resulted on Benoni, Hawley, Jonathan, Melon, and White Pippin.

Inoculations with reisolated cultures by hypodermic injection on White Pippin, Jonathan, and Melon were also successful. The organism isolated from infections agreed in all characteristics, morphological and cultural, with the one used for inoculation. Checks to correspond with the three methods of inoculation described above remained healthy throughout the season.

ROUGH-BARK OR SCURFY-BARK CANKER OF APPLES

Description of the disease

What might be called the quiescent stage of the scurfy-bark disease occurs as patches of roughened scaly bark which somewhat resemble blotch canker (*Phyllosticta solitaria* E. and E.), but differ from it in showing no blotch pycnidia and usually no regularity of cracking up and down or across the limb (fig. 2, A). These roughened patches vary greatly in size from those covering only a few square centimeters to those covering the whole side of a limb for a meter or more. They are usually found on the north side of a limb and with few exceptions are bordered by a pimpled

lumpy ridge slightly more brown or reddish brown than the healthy bark around them.

The active stage of the disease, occurring in early spring, is characterized by a loosening and sloughing off of the outer bark, quite unlike anything that takes place in any other canker known to the writer. It reaches

FIG. 2. SCURFY-BARK CANKER ON WHITE TRANSPARENT (DWARF) APPLE

A, *B*, Pimpled lumpy ridge at the edge of roughened areas. *C*, typical roughening, with an area exposed by deep-peeling, at *x*. *D*, the more common type of roughening, without marked peeling of affected bark.

its extreme form on certain varieties of dwarfs but has been observed on Logan, Munson, Ben Davis, and several others. For convenience of reference it wi l be known in this paper as the "deep-peeling" type of the disease.

In the development of this active stage, the first change visible from the outside is the formation of narrow cracks, 1.0 to 1.5 mm. deep by 5 to 20 cm. long, about 1 cm. outside of the pimply ridge referred to above

(fig. 3, *A*). Inside of these cracks the bark is found loosened—as early as March 21, 1916—in a layer about 2 mm. deep. It is easily peeled off and if this be done there is revealed a spongy layer of giant cells (fig. 3, *B*) about 1 mm. thick, which is white or greenish white at first, but which

FIG. 3. SCURFY-BARK CANKER

A, Limb of White Transparent (dwarf), natural size, showing typical loosening of bark by "deep-peeling" type of scurfy-bark canker. Photograph made April 25, 1916. *B*, Portion of another limb, with loosened layer removed, showing spongy layer beneath. Photograph made April 25, 1916. × 1.5.

C, Limb of Count of Wick (dwarf). Photograph made August 19, 1916, three months after inoculation with strain A 9 isolated from diseased bark. Natural size.

soon oxidizes to a pale brown and later to a dark brown color. Under natural conditions the loosened outer layer begins to dry and curl slightly within a few days after the cracks are formed; as a consequence the cracks widen, air circulates more freely under the loosened layer and the spongy layer dries down. Within two weeks from the time the cracks first show there is usually no spongy layer to be found and the loosened layer has become dried, curled fragments which break off almost at a touch. Many varieties show no wholesale loosening of the outer bark, but merely a

scaling off of small patches without the formation of a definite spongy layer. Sometimes the dry, brown vestiges of such a layer can be found, sometimes not. Possibly in such cases it develops slowly and progressively from one point to another, loosening the bark only a little at a time and drying down almost immediately.

No detailed studies of the diseased tissues have been made. A preliminary survey of both free-hand and microtome sections shows the presence of peculiar spots or pustules like those described by Hewitt[5] in his work with a new disease of apple bark in Arkansas and by the writer[6] in work with what is almost certainly the same disease, known at this station for over ten years under the name of pimple canker. It remains to be proved, however, whether this disease is a phase of or in any way related to the true scurfy bark canker. The most that can be said at present is that they show considerable morphological similarity and are often though not always associated on the same tree.

Besides the deep-peeling type of scurfy-bark canker there sometimes occurs a "shallow-peeling" type, in which only the epidermis is loosened. A spongy layer is formed here also, but it is thinner and more evanescent than in the deep-peeling type.

Occurrence of the disease

The disease occurs on such standard varieties as Ben Davis, Jonathan, Logan, White Winter Pearmain, Beach, Stayman Winesap, Munson, and Marsh, but much more severely on certain dwarf varieties.

The writer has found the disease in numerous orchards in southern Missouri and has received specimens of it from perhaps a dozen localities scattered over the state. No information is at hand as to is occurrence in other states, unless the trouble investigated by Hewitt in Arkansas be considered a phase of it. Affected trees are not quickly killed as in the case of Illinois canker—caused by *Nummularia discreta*—but there is no doubt that the peeling off of fresh layers of bark every spring is definitely injurious to the tree, aside from the opportunity given for entrance of canker fungi and various bark insects.

Cause of the disease

The disease seems to be caused by the same organism as that of the fruit spot. Poured agar plates, using material from (1) the deep lying

[5] Hewitt, J. Lee. An unknown apple disease. Arkansas Exp. Sta. Bul. **122**: 481–491. 1912.

[6] Rose, Dean H. Report of the Pathologist. Missouri State Fruit Exp. Sta. Rept. **1913–14** (Bul. 24): 30. 1914.

spongy layer, (2) the pimply ridge at the edge of roughened areas, (3) areas exposed naturally the preceding season when the loosened layer peeled off, (4) the spongy layer under loosened epidermis, gave practically pure cultures of an organism very similar, morphologically, to the blister-spot organism. Extensive cultural studies show, however, that instead of one organism there are two different ones or possibly two closely related but distinct varieties. One of them, represented by five strains, shows great similarity in morphological and cultural characteristics to the blister-spot organism; the other, represented by fifteen strains, resembles the blister-spot organism morphologically but differs from it in cultural characteristics. It liquefies gelatin rather rapidly, it produces a green fluorescence on nutrient agar and it begins to clear litmus milk inside of twenty-four to thirty-six hours. The first and second of these are not characteristic of the blister-spot organism, while the last is characteristic only of three strains.

Inoculations

Inoculation of bark with the rapidly liquefying bark organism produced swollen spots 1 to 2 mm. high and covering an area of roughly 1 sq. cm. (fig. 3, C). At these swellings the typical signs of the disease were reproduced, in miniature, and an organism was recovered which agreed in cultural and morphological characteristics with the one used for inoculation. The checks showed only a slight swelling. Typical blister spots on the fruit were produced by inoculating Jonathan and Melon apples with both bark organisms and these in turn were recovered from the lesions produced. While such evidence is strongly suggestive it is not final proof, and more work is necessary before the true relation between the fruit disease and the bark disease can be discovered.

In table 1 are shown the results of comparative tests made with the blister-spot organism and the rapidly liquefying bark organism.

A similar comparison between the bark and the blister-spot organism on the one hand and *Pseudomonas fluorescens* on the other show so many differences that the two former must be considered entirely distinct from the latter.

Further work on these diseases should include (1) a continuation of cultural studies of the blister-spot organism and the two bark organisms, (2) cross-inoculations from bark to fruit and from fruit to bark, (3) a study of the time and mode of infection and (4) a study of the microscopical characteristics of healthy and diseased tissues.

TABLE 1

Comparison of the blister-spot organism and the bark organism

	BLISTER-SPOT ORGANISM[1]	BARK ORGANISM[2]
Agar plates	Whitish colonies, slightly bluish in transmitted light; medium not greened	Whitish colonies, slightly bluish in transmitted light; medium slightly greened
Agar slant	Growth rather slow, no fluorescence	Growth rather slow, fluorescence marked
+ 10 bouillon	Clouding and pellicle; one strain showed faint green fluorescence in 2 weeks	Clouding; green fluorescence after 1 week; four strains showed pellicle
Gelatin stab	Liquefaction slow, begins in 2–3 days. Complete in 12–14 weeks. No fluorescence	Liquefaction more rapid, begins in 24 hours, complete in 2 months. Slight fluorescence after 1 week
Neutral gelatin stab	Liquefaction slow. Slight fluorescence	Liquefaction more rapid. Marked fluorescence
Litmus milk	Alkaline reaction for 10 days, then slow increase in acidity up to 40 days. Medium cleared in 2–3 weeks; final color blue throughout. Banded appearance produced by 3 strains	Slight alkaline reaction at top in 2 days, acid reaction below. Acidity increases as digestion proceeds. Banded appearance resulting in clearing in 3–4 weeks. Final color greenish blue above, buff below
Sterile milk	Clearing begins in from 7–10 days, complete in 17–20 days	Clearing begins in 36 hours, complete in 3–4 weeks. Green fluorescence
Glycerin agar	Growth vigorous, elevated, contoured to rugose. Moderate fluorescence	Growth vigorous, elevated, contoured to rugose. Fluorescence more marked than for blister-spot organism
Uschinsky's solution	Clouding moderate; bluish green fluorescence. Pellicle of pseudo-zoogleae-like fragments	Same as for blister-spot organism except fluorescence more marked with some strains
Nitrate reduction	None	None
Indol test	No indol in 20 days	Indol present in 10 days
Ammonia test	Ammonia produced	More ammonia produced than by the blister-spot organism
Resistance to sunlight	Average of 98 per cent killed after 10 minutes exposure	Average of 50 per cent killed after 10 minutes exposure
Flagella	Bipolar; one to several	Bipolar; one to several

[1] Under this heading are included also the slow liquefiers from bark.

[2] Under this heading are included only the rapid liquefiers from bark.

METHODS AND MEDIA

As standards for methods and for the making of media the writer has followed directions given in Erwin F. Smith's Bacteria in Relation to Plant Diseases, Vol. I, and Eyre's Bacteriological Technic, 2nd Ed., except where noted otherwise in the text.

SUMMARY

1. In the foregoing paper is described a bacterial disease of apples, no mention of which has been found in phytopathological literature.

2. By isolation, cultural, and inoculation work, it is proved that this disease is caused by a motile organism, which liquefies gelatin slowly, and belongs to the green-fluorescent group of bacteria.

3. Because of the blister spots produced by this organism on the surface of apples, the name *Pseudomonas papulans* is proposed.

4. Description is also given of a rough-bark or scurfy bark canker from which has been isolated an organism also belonging to the green-fluorescent group.

5. Evidence is presented that there are really two varieties of the bark organism, one of which has all of the cultural characteristics of the blister-spot organism, including slow liquefaction of gelatin, while the other differs from it in several important particulars, including rather rapid liquefaction of gelatin.

6. Inoculation of healthy apple bark with two strains of the rapidly liquefying bark organism produced small lesions which showed the typical cracking loose of diseases from healthy bark, and in several cases the lumpy appearance characteristic of the early stages of the scurfy-bark canker.

7. An organism agreeing in cultural reactions with the one used for inoculation has been recovered from these lesions.

8. Typical blister spots have been produced by inoculation of both types of bark organisms into healthy apples. From these spots the organisms used for inoculation have been recovered.

9. A preliminary comparative study of the cultural characteristics of the blister-spot organism and the two bark organisms suggests that the difference between them are differences of degree rather than of kind. That is, that all three are possibly merely varieties of one species. More work is necessary, however, before this question of relationship can be settled.

MISSOURI STATE FRUIT EXPERIMENT STATION
MOUNTAIN GROVE, MISSOURI

THE PATHOGENIC ACTION OF RHIZOCTONIA ON POTATO

H. T. Güssow

WITH ONE FIGURE IN THE TEXT

It appears that satisfactory evidence has been lacking clearly demonstrating the pathogenic action of Rhizoctonia (*Corticium vagum* B. & C.) on various host plants, particularly the potato.

The attention which the well known disease has received in the new world and more recently in the old, left, in my opinion and in that of quite a number of other investigators, several important points unexplained. Every pathologist fully recognized the symptoms of this disease which have come to be regarded as typical Rhizoctonia infection. The unmistakable folding of the leaves of the growing plant, together with the brown stem lesions so frequently described, in other instances, or in addition perhaps, the formation of aerial tubers and peculiar smallness of subterranean tubers, are now well known as general symptoms resulting from an attack of Rhizoctonia.

Indeed, the folding of the leaves associated with this disease differs greatly from the curling of the leaves of plants affected with leaf roll, particularly noticeable is this difference when examining the lower leaves of an affected plant. Where, however, doubt existed, the presence of stem lesions was looked upon as final proof of a Rhizoctonia infection.

I must confess, however, that the often surprising scarcity and apparent superficiality of these lesions, nay, often enough their entire absence in what was otherwise unmistakably a plant infected with Rhizoctonia and not with leaf roll, frequently caused me surprise and certainly failed to readily convince the farmers on the occasion of field demonstrations of the correctness of the diagnosis—which at times I shared much against my own belief.

For some time I have endeavored to discover the true pathogenic action of this fungus on the host plant. My colleague, Drayton,[1] demonstrated to my satisfaction the profuse permeation of the tissues of and surrounding the lesions, but even that failed to convince me entirely of the cause of the characteristic symptoms, which statement is not intended to infer

[1] Drayton, F. L. The Rhizoctonia lesions on potato stems. Phytopath. **5**: 59. 1915.

that I doubted Rhizoctonia to be associated with the same. But from what evidence was available, the actual injuries caused by the stem lesions were so infinitesimal that it was felt the true injury is done elsewhere, and what we did observe was the result of such unlocated but far more serious injury.

A careful study of diseased plants in the field revealed at first little or no additional clues. Lesions were sometimes present where the leaves were folded, the tubers were covered with more or less numerous lumpy fungous masses, indeed the roots often showed the well known pseudosclerotia. Pot experiments showed the presence of sclerotia on rootlets more abundantly than was the case in the field, and yet while abundant superficial and lesser amounts of intracellular hyphae of Rhizoctonia were found on microscopical examination, the evidence of an all round general soundness of the underground parts examined still left the seat of the injury undetermined.

On careful examination of the root system of a plant clearly affected with Rhizoctonia and no other disease, that had been pulled up from loose sandy soil, or had been lifted with care by means of a fork or spade, one factor at last attracted my attention, which later led to interesting observations. This was the almost entire absence of the fine fibrous rootlets, so common in sound plants. Surely such rootlets must have been present originally? When examining thereafter plants in various stages of infection one could observe a corresponding absence or presence of finer rootlets according to the amount of disease present. Of course, in this determination care is necessary, but after some experience one cannot but recognize the existing relation of rootlets to degree of disease.

What if the fungus acted upon the roots of the growing plant similar to the way in which it does on the roots of Rhizoctonia-infected tubers sprouted in a closed stender dish? The disastrous effects of the fungus at the early stages of growth are sometimes so pronounced as to kill off growth altogether; this is a well known fact.

Let us bear in mind that in a potato field we find many stages of severity of Rhizoctonia infection, from total "misses" to one, two or more shoots clearly affected up to the case where the plant bears plenty of aerial tubers and numerous little potatoes underground from which the popular name "little potato disease" has sprung. Aerial tubers have been commonly associated with Rhizoctonia, they are perhaps exclusively manufactured from material produced by the leaves, a comparatively slow process, but always indicating impaired root function in plants where they may be considered abnormal. Aerial tubers naturally may occur from any cause cutting off or interrupting root function, but only when such interruptions are gradual. We have, therefore, no aerial tubers in the black

but the roots infected with sclerotia remain in the ground, since they are not pulled up by the digger or are at any rate returned to the ground. With the diminishing food supply in these roots, sclerotia develop ready for subsequent attacks. This observation also accounts largely for the soil contamination and the persistence of the organism in land once infected. It also indirectly suggests a new means of control, viz., the prevention of infection by cultural methods or the application of fertilizers producing vigorous plants in the first instance and aiding in the production of a generous supply of new feeding roots.

CENTRAL EXPERIMENTAL FARMS
OTTAWA, CANADA

leg disease, where the cutting off of supplies is rather sudden. All these symptoms are the logical results of the absence of the abundant feeding roots. Roots are present in all growing plants, otherwise the plants would have died; small and fine roots are less in evidence in affected plants while a generous supply exists in healthy strong plants.

Deductions—however logical they may be—still are hypotheses and hypotheses are not facts, but the accompanying plate will provide some foundation for the observations recorded and may stimulate wider researches on this point than have been made so far. I am satisfied from the observations made, that the destruction—often very gradual—but very persistent all the same, of all or many of the feeding roots of the potato plant accounts for every one of the symptoms associated with this disease. The lesions which have so often been recorded are evidently not of serious consequence, as indicated by their general superficiality and frequent entire absence. In some instances indeed these lesions are not due to Rhizoctonia at all, but to *Actinomyces scabies* Güssow, which I hope to show in another paper, when they afford easy resting places in the unprotected superficial cells for the mycelial masses of Rhizoctonia shown in Mr. Drayton's photo-micrographs, as well as for the permeation of the hyphae into the interior, which, as must have been noticed, is not accompanied by any prominent injurious action upon the cells invaded. A study of Mr. Drayton's slides clearly confirms this observation as well as the photographs made from them which are accessible to our readers.

The pathogenic action is as follows: We are aware of the very profuse growth of mycelium of Rhizoctonia, particularly in the dark, as also of the production of enormous quantities of pseudo-sclerotia on roots and tubers. Whether the sclerotia are left over in the soil from preceding potato crops or other host plants, or whether they have been introduced by untreated infected seed potatoes—(and what "farmers' run" potatoes are not infected?) does not matter much. The tips of the fresh rootlets soon fall a victim to the invading mycelium, the root cap being undoubtedly the most vulnerable point and soon the short roots have been destroyed, the mycelium meanwhile reaches older rootlets, which it much more rarely destroys, though that has occurred, but where the mycelium frequently produces resting mycelial masses from which invading hyphae issue almost simultaneously with new rootlets which are produced by the plant in its effort to reestablish its resources. This process goes on gradually and slowly or more rapidly depending naturally upon the vigor of the plant. Finally the persistent efforts of the fungus result in decreasing yields, in frustrating the growth of the tubers, because of lack of food supplies from the roots, and eventually in the production of aerial tubers. Meanwhile harvest-time has arrived, what tubers are there, are harvested.

but the roots infected with sclerotia remain in the ground, since they are not pulled up by the digger or are at any rate returned to the ground. With the diminishing food supply in these roots, sclerotia develop ready for subsequent attacks. This observation also accounts largely for the soil contamination and the persistence of the organism in land once infected. It also indirectly suggests a new means of control, viz., the prevention of infection by cultural methods or the application of fertilizers producing vigorous plants in the first instance and aiding in the production of a generous supply of new feeding roots.

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OTTAWA, CANADA

SYNTHETIC CULTURE MEDIA FOR WOOD-DESTROYING FUNGI[1]

ERNEST J. PIEPER, C. J. HUMPHREY AND S. F. ACREE

Wood-destroying fungi grow readily on many of the ordinary culture media which have as their principal ingredients malt extract or plant decoctions and meat extracts, usually hardened with agar-agar or gelatin. As an example of such media, a malt extract preparation of the following formula has proved very satisfactory for general culture work:

Extract of 1 pound lean beef[2] in distilled water	1000 cc.
Malt extract	25 grams
Agar-agar	20 grams

This has found a wide use in Europe and has been employed for much of the routine work in the pathological section of the Forest Products Laboratory. In certain lines of investigation, however, such as the testing of the toxicity of chemical substances, and in comparative tests on the physiological behavior of wood-destroying fungi, this medium has the serious disadvantages of being chemically complex and variable. Its composition and constitution depend upon the nature of the meat and malt extract, the method of preparation and the duration of standing, and physically it may be variably colloidal. The possibility of chemical or physical combination of certain preservatives with the highly complex organic compounds and also the coagulation of the latter by electrolytes are of extreme importance also in toxicity work.

In the present work an attempt was made to prepare a synthetic medium which would support a growth of wood-destroying fungi at least as good as that on the malt extract agar, and which at the same time would be composed of as simple constituents as possible. The medium could then be duplicated at any time, by any investigator, provided the chemicals used were of the same standard of purity.

For satisfactory growth of wood-destroying fungi a culture medium must have, in addition to certain simple inorganic salts, the necessary compounds to furnish both nitrogen and carbon in a form readily available to the fungus. Ammonium salts, nitrates and asparagin or its salts have

[1] The present paper is one of four prepared by the junior author (Pieper) in partial fulfillment of requirements for the degree of Doctor of Philosophy in the University of Wisconsin.

[2] Later tests in this laboratory indicate that beef is of little or no advantage, in many cases it somewhat retards the growth of wood-destroying fungi.

frequently been used in synthetic media as a source of nitrogen, while various carbohydrates have been used as a source of carbon.

The first step in this work was to select a nutrient solution of inorganic salts with di-ammonium phosphate as the source of nitrogen. This solution was used as the basis for testing the nutrient value of various carbohydrates and consisted of the following:

Di-potassium phosphate (K_2HPO_4)	4 grams
Di-ammonium phosphate ($(NH_4)_2HPO_4$)	2 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2 grams
Agar-agar (powdered)	15 grams
Distilled water	1000 cc.

Varying concentrations of the following carbohydrates, usually 20 or 40 grams in a liter of the nutrient solution, were used: Lactose, maltose, cane-sugar, galactose, glucose and glucosamin. Growth of the fungus[3] was compared with that on malt extract agar as a standard.

The organism grew fairly well in every case. Cane-sugar and glucose, however, gave the most favorable indications, the growth on the cane-sugar being slightly more favorable. Since, however, the growth might be changed by using other nitrogen sources in addition to di-ammonium phosphate, it was decided to continue the use of both sugars in further work.

The next step was to obtain a nitrogen source more available to the fungus than di-ammonium phosphate, so the following substances were tested by adding 2 and 4 grams, respectively, to a liter of the nutrient solution containing forty grams of cane-sugar: Asparagin, sodium asparaginate, ammonium asparaginate, caffein, guanidin carbonate, glycin, leucin, creatinin and betain. With 0.2 and 0.4 per cent of caffein and guanidin carbonate, respectively, no growth of the fungus occurred, but in all the other cases a fairly good development was secured. The best growth was, without doubt, obtained with asparagin and its sodium and ammonium salts. The fact that asparagin alone might be a source of available carbon for fungi was considered, but experiments showed that practically negative results were obtained in the absence of a sugar.

Glucose was next substituted for cane-sugar while using asparagin and di-ammonium phosphate as a source of nitrogen. The character and rate of growth was the same as that obtained with cane-sugar. It has been shown[4] that not all fungi contain an enzyme which will hydrolyze cane-sugar, hence the substitution of glucose would presumably be an advantage, and cane-sugar was therefore discarded.

[3] *Fomes annosus* was used in all the preliminary tests.

[4] Boeseken, J., and Waterman, H. Akad. Wetensch. Amsterdam, **20**: 548. 1911. Abstract in Bot. Gaz. **59**: 413. 1915.

On the basis of these experiments a culture medium of the following composition was selected for further test:

Glucose ($C_6H_{12}O_6$), powdered	40.00 grams
Di-potassium phosphate (K_2HPO_4)	4.00 grams
Asparagin ($C_4H_8O_3N_2$)	4.00 grams
Di-ammonium phosphate ($(NH_4)_2HPO_4$)	2.00 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2.00 grams
Calcium carbonate ($CaCO_3$)	0.25 gram
Calcium chloride ($CaCl_2$)	0.10 gram
Agar-agar (powdered)	15.00 grams
Distilled water	1000.00 cc.

It is evident that this medium is synthetic except for the agar-agar[1] which is used as a solidifying agent. Its nutritive properties for four important wood-destroying fungi were determined. As a comparison, similar tests were made on another medium differing only in the substitution of Witte's peptone for asparagin and di-ammonium phosphate. Although this latter medium is not strictly synthetic, still a definite grade and purity of the peptone can be obtained from a reliable source. For many purposes such a medium might be found of considerable advantage, as has been shown by tests on *Fomes annosus* and *Fomes pinicola* reported later.

In preparing the synthetic medium, the mixture was heated in a 1.3-liter flask in a water bath to prevent charring. It was then poured into test-tubes as quickly as possible, and well stirred during the operation to avoid losing any precipitate that was formed. The tubes were then plugged with cotton and sterilized in live steam (100°C.) without pressure for thirty minutes on three successive days. A very slight precipitation was found in the bottom of the tubes after sterilization. This was probably a mixture of calcium and magnesium phosphates and carbonates. As small amounts of calcium salts seem to increase the vigor of the growth, it is advisable to retain this sediment by thoroughly shaking the tubes before pouring into petri dishes.

The Witte's peptone medium was prepared in the same manner as the synthetic and was composed of the following substances:

Glucose ($C_6H_{12}O_6$), powdered	40 00 grams
Di-potassium phosphate (K_2HPO_4)	4 00 grams
Witte's peptone	4 00 grams
Magnesium sulphate ($MgSO_4.7H_2O$)	2 00 grams
Calcium carbonate ($CaCO_3$)	0 25 gram
Calcium chloride ($CaCl_2$)	0 10 gram
Agar-agar powdered	15 00 grams
Distilled water	1000 00 cc.

[1] Colloidal silicic acid has been used by some workers in place of agar-agar and might be of advantage here but the writers have not experimented with it.

The malt extract medium used for comparison was made as previously indicated. It was tubed and sterilized in the same manner as the others.

All these media were tested with four wood-destroying fungi. The tables give the growth of these at 25°C. over varying periods up to twenty-eight days.

From the results it is seen that the synthetic culture medium which produced the best results gives a good growth and compares favorably with the malt-extract medium. Perhaps by continued cultivation the fungi may gradually become adapted to the new medium, giving then better results than were obtained in this investigation.

The Witte's peptone medium is especially good for the growth of *Fomes pinicola* and *Fomes annosus*. With *Lenzites sepiaria*, and especially with *Stereum frustulosum*, it gave less favorable results than the other two.

By additional work it may be possible to improve the synthetic medium further so that it will give a better and more uniform growth than here described. Such a medium would be very valuable both for toxicity work and general physiological experimentation with wood-destroying fungi.

In addition to the media for which the formulae are given in the present paper sixteen other preparations were tried. The proportions given are for 1000 cc. distilled water and 1.5 per cent agar. None of these were as satisfactory as those reported above.

1. Cane sugar, 40 grams; ammonium asparaginate, 4 grams; magnesium sulfate, 2 grams.

Gave thick but slow growth for *Fomes pinicola;* with *Fomes annosus* gave thin growth, covering the plate in two weeks.

2. Fraenkel and Voges' Solution.[6]

Gave very poor growth.

3. Fermi's Culture-Fluid.[6]

Gave poor thin growth; plate not covered in three weeks.

4. Uschinsky's Solution.[6]

Gave poor thin growth; plate not covered in three weeks.

5. Modified Uschinsky's Solution.[6]

Gave very poor growth.

6. Hasselbring's Solution.[7]

7. Cane sugar, 40 grams; glycerin, 40 grams; asparagin, 4 grams; magnesium sulfate, 2 grams; di-potassium phosphate, 4 grams; di-ammonium phosphate, 4 grams.

Gave best growth for *Fomes annosus;* thin growth for *Fomes pinicola;* thick but slow growth for *Fomes applanatus, Lenzites sepiaria* and *Stereum frustulosum.*

[6] See E. F. Smith, Bacteria in relation to plant diseases, Vol. I, p. 197, 1905.

[7] Glucose, 1 gram; ammonium nitrate, 1 gram; di-potassium phosphate, 0.5 gram; magnesium sulfate, 0.25 gram.

TABLE 1

Radial growth in millimeters of Fomes annosus in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS		
		7 days	10 days	14 days
Synthetic	1	21–23	30–34	40–42*
	1′	23–24	31–35	40–48*
Peptone	2	23–24	35–37	42–44*
	2′	24–26	33–39	40–42*
Malt-extract	3	24–25	29–34	40–43*
	3′	26–28	30–35	41–45*

Character of growth:

Synthetic. Slightly more fluffy and slightly less dense than on malt-extract, although the color was the same.

Peptone. About the same as on synthetic medium but not quite as dense.

Malt-extract. Dense, creamy, fluffy growth.

The growth of *Fomes annosus* on synthetic medium and peptone medium was tested for acidity with litmus and found to give a slight acid reaction after fourteen days growth.

*Surface of medium entirely covered.

TABLE 2

Radial growth in millimeters of Fomes pinicola in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS			
		7 days	10 days	14 days	21 days
Synthetic	1	24–28	29–32	33–35	Surface covered
	1′	24–26	27–33	30–35	Surface covered
Peptone	2	21–22	31–32	Surface covered	
	2′	21–22	31–32	Surface covered	
Malt-extract	3	26–27	32–33	Surface covered	
	3′	25–27	32–34	Surface covered	

Character of growth:

Synthetic. Thin and striated, color same as on malt-extract medium

Peptone. Very fluffy and just as dense as on malt-extract medium

Malt-extract. White, dense, fluffy growth.

TABLE 3

Radial growth in millimeters of Lenzites sepiaria in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS				
		7 days	10 days	14 days	21 days	28 days
Synthetic	1	21–22	30–31	33–37	Surface covered	
	1′	22–23	31–32	37–38	Surface covered	
Peptone	2	17–20	28–30	34–35	37–39	Surface covered
	2′	16–20	29–31	35–36	36–39	Surface covered
Malt-extract	3	17–18	27–30	33–35	Surface covered	
	3′	17–20	29–30	36–38	Surface covered	

Character of growth:

Synthetic. Margin of growth thin with much sub-growth; more fluffy towards center than on malt-extract; color not as dark as on malt-extract medium.

Peptone. Growth poor in appearance; color same as on malt-extract medium.

Malt-extract. Fairly dense; dirty brown color.

TABLE 4

Radial growth in millimeters of Stereum frustulosum in various media at 25°C.

MEDIUM	NUMBER OF PETRI-DISH	RADIAL GROWTH OF FUNGUS IN MILLIMETERS				
		7 days	10 days	14 days	21 days	28 days
Synthetic	1	9–12	14–16	29–33	Surface covered	
	1′	10–11	15–19	28–34	Surface covered	
Peptone	2	4–5	5–6	8–9	9–10	10–11
	2′	7–9	9–11	10–13	12–13	13–14
Malt-extract	3	11–16	21–22	26–28	30–36	Surface covered
	3′	13–14	22–23	27–29	36–37	Surface covered

Character of growth:

Synthetic. Very fluffy, dense and creamy; slightly more brown in color than on malt-extract medium.

Peptone. Poor growth; deep orange-brown color.

Malt-extract. Fluffy growth; slight yellow tint.

8. Differs from No. 7 in substitution of 40 grams galactose for the cane sugar.

Gave much slower growth than No. 7; plate not quite covered in three weeks.

9. Differs from No. 7 in substitution of 40 grams lactose for the cane sugar.

Gave slow growth, not as good as No. 8; plate not covered in three weeks.

10. Cane sugar, 40 grams; glycin, 4 grams.; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Gave good growth; almost as rapid as on malt-extract agar, but appearance not as good as on No. 1.

11. Differs from No. 10 in substitution of 4 grams creatinin for the glycin.

Gave fair growth; plate covered in three weeks.

12. Differs from No. 10 in the substitution of 4 grams. caffein for the glycin.

No growth in three weeks.

13. Differs from No. 10 in the substitution of 4 grams guanidin carbonate for the glycin.

Gave no growth in three weeks.

14. Cane sugar, 20 grams; glucosamin, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Fairly good growth, but less dense than on malt-extract agar; plate covered in two weeks.

15. Glucose, 25 grams; d and l-leucin, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Plate covered in two weeks but growth thinner than on No. 10.

16. Lactose, 32 grams; betain, 4 grams; di-potassium phosphate, 4 grams; magnesium sulfate, 2 grams.

Growth fair; plate not quite covered in three weeks.

LITERATURE

As practically all of the literature dealing with synthetic culture media, as far as the writers have investigated, has reference to its adaptability to the growth of bacteria or molds and as these organisms apparently react to the media in a different manner than the wood-destroying Hymenomycetes upon which this study was conducted no bibliography is appended.

INVESTIGATIONS IN FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY
IN COOPERATION WITH THE FOREST PRODUCTS LABORATORY,
MADISON, WISCONSIN

PHYTOPATHOLOGICAL NOTES

Apple scab on the twigs. Does the apple scab (*Venturia pomi* (Fr.) Wint.) pass the winter on the twigs, which thus become a source of infection? This is a question frequently presented to the American plant pathologists. The presence of the organism in the twigs has been reported by a number of workers, but the first satisfactory data bearing on the probabilities of this being a source of infection were presented by Morse and Darrow in 1913.[1] They gave a very satisfactory review of the literature of the subject, which will therefore be omitted in this discussion.

In the latter part of April, 1916, the attention of the writers was called to a number of diseased apple twigs from Freehold, Monmouth County, New Jersey. A careful examination showed that the organism was *Venturia pomi*, and that the conidia which were present in great numbers were viable.

The twigs showed an abundance of infection for several inches and the bark was split, thus giving them a very ragged appearance. Directly beneath the ruptured bark were dense masses of stromatic growth, which protruded through the openings and produced an abundance of conidiospores, which were short and irregular and rested on still shorter, in fact almost equilateral, irregular basal cells. The spores were typical and agreed in shape and size with those obtained from other sources, and grew readily in culture. We are unable to say whether these spores were formed in the fall of 1915 or the spring of 1916, but it is very evident that the organism survived the winter in the twigs.

The writers did not have an opportunity to examine the trees, but Mr. W. B. Duryee, Jr., the County Farm Demonstrator who sent the twigs to the Station, reported the disease in abundance. The twigs submitted to us carried an abundance of the organism, and the infection extended from the tip back some 12 to 15 inches, but was most severe near the tip.

Many of our New Jersey apple growers cultivate their orchards so

[1] Morse, W. J. Spraying experiments and studies on certain apple diseases in 1913. Maine Agr. Exp. Sta. Bul. 223. 1914.

Morse, W. J. Spraying experiments and apple diseases. Maine Agr. Exp. Sta. Bul. 252. 1916.

Morse, W. J. and Darrow, W. H. Is apple scab on young shoots a source of spring infection? Phytopath. **3**: 266–269. 1913.

thoroughly that fallen leaves are very scarce in the spring of the year. However, these same orchards will some times produce an abundance of diseased fruit. Although we fully realize that a severe infection may result from a small source, we have for some time been inclined to believe that there must be some source of infection other than the ascospores formed on the leaves of the preceding year.

MEL. T. COOK AND C. A. SCHWARZE

On using an ether freezing microtome in warm and damp weather. Most persons who use an ether freezing microtome may remember that during the warm and humid days of midsummer and early fall there is likely to be considerable difficulty in freezing material, when at other times little or no difficulty is experienced. During the very damp and rainy days of early summer (1916) much difficulty of this sort was experienced in the Laboratory of Forest Pathology, Providence, R. I. At times it was absolutely impossible to freeze the preparation. This led to some experimenting on the part of Mr. N. O. Howard, Collaborator, and the writer, which finally resulted in overcoming the main difficulty in a very simple and efficient manner. The apparatus consisted merely of a wide-mouthed, eight- or ten-ounce bottle containing anhydrous calcium chloride, which was inserted between the pressure tank and the atomizer. This bottle was connected so that the air from the tank passed through a glass tube in the stopper to the bottom of the bottle, and thence up through the mass of calcium chloride to another glass tube which connected with the atomizer by means of a rubber tube.

The calcium chloride was broken into small pieces and packed into the bottle, but not so tightly as to prevent the long glass inlet tube being worked down through the mass as the stopper was inserted. The rubber stopper was tied securely in place so as to prevent its being blown out by an excess of air pressure.

After a few days of occasional use the calcium chloride usually showed signs of deliquescence through absorption of moisture from the air. When this deliquescence became rather pronounced, the calcium chloride was placed in a small "fry-pan" and heated until it was again entirely dry and hard. After it had cooled sufficiently to handle it was broken up into small pieces and put back in the bottle while still warm. The same calcium chloride, which cost but a few cents when purchased, has now been in use for more than six months. When the freezing microtome was used several times each day the calcium chloride had to be dried about every week or ten days.

Although the apparatus described may be considered as a somewhat crude affair, it has worked very efficiently for more than six months and

has given no indication that its efficiency would not continue indefinitely. Without doubt a more finished apparatus of still greater efficiency would result from using a regulation chemical dehydrating apparatus.

Although this method of manipulation usually prevents the formation of snow on the under side of the freezing disk—which delays freezing—it does not always prevent it. Recent experiments by Mr. Howard show that this difficulty can be prevented entirely by placing several small lumps of anhydrous calcium chloride in the ether bottle, or, better, in the ether can itself as soon as it is opened, and letting it stand for half a day or a day before using. This withdraws the small amount of water in the ether, which apparently is partly responsible for the formation of the snow.

In using ether for freezing sections we have always found it necessary to filter the ether before it reaches the atomizer and also to avoid using rubber in contact with it. The ether intake tube in our microtome has an inside diameter of less than 2 mm., and filtering is very easily accomplished by thrusting a small wad of cotton into the end of the tube. This wad of cotton also is extremely useful in regulating the supply of ether going to the atomizer, as, with a little experience, the supply can be increased or decreased almost at will by using respectively a loose wad of cotton or a compact one. Of course the cotton used for filtering should be renewed whenever it shows any tendency to become clogged.

This note is offered for publication with the thought that other workers who have had similar difficulties in freezing material with ether might like to know that such difficulties can be overcome so easily.

J. FRANKLIN COLLINS

Note on Xylaria polymorpha and X. digitata. The recent article by Fromme and Thomas[1] on a root-rot disease of the apple in Virginia, in which the causal organism is provisionally referred to some species of Xylaria, may be further substantiated by the following observations.

In 1906, near Scottsburg, Indiana, the writer collected mature specimens of *Xylaria polymorpha* from diseased areas in living roots of a four-years-old apple tree of the variety Winesap. The following year this tree died and was pulled up. The conidial stage of the fungus was afterwards noted on the diseased roots. On October 5, 1908, in the same orchard *Xylaria digitata* was collected from the roots of a six-years-old pear tree which had died from some unknown cause. In the writer's herbarium are two other collections of *Xylaria digitata*, made at Priest River, Idaho, from decayed areas in living roots of *Populus trichocarpa* and *Cratægus*

[1] Science n. s. **45**: 93. 1917.

douglasii. The roots of the former were partially decayed, but not in the same part, by *Fomes applanatus*, and those of the latter by a species of Fomes[1] peculiar to this tree.

JAMES R. WEIR

Puccinia triticina Erikss. Leaf-rust of winter wheat causes damage in Kansas. It is generally considered that the leaf-rust of wheat due to *Puccinia triticina* Erikss. is not serious enough to cause any appreciable damage to the crop, at least publications indicate that an attack of leaf-rust in May or June does not produce any marked effect on the yield.

Observations by the writer the past season showed that the leaf-rust in some fields in Kansas was very abundant, and that its occurrence was not confined to the foliage but that the "necks" of the wheat were vigorously attacked by this rust. Careful observations and examinations of such fields showed that no other factors could have been responsible for the poor quality of the grain and the reduced yield. The yield of one variety in particular, a pure line winter wheat grown in Kansas and called P 706, was reduced 38 per cent, according to yield data furnished by the Department of Agronomy. The fields showing the effect most were those which had been planted late. It is thought that this is partially responsible for the large percentage of leaf-rust.

The percentage of infection on the "necks" of the wheat, as estimated by the newly adopted scale for estimating rust percentages of the Office of Cereal Investigations, Department of Agriculture, was 10 to 25 per cent, while the foliage of the above-mentioned variety generally showed 100 per cent of infection.

It is believed by the writer that the leaf-rust of winter wheat in Kansas can under favorable conditions cause considerable damage and that too little stress has been given in literature to the importance of this rust.

L. E. MELCHERS

Early discovery of white pine blister rust in the United States. There has recently come to the attention of the writer the fact that a specimen of white pine blister rust was collected on white pine (*Pinus strobus* Linn.) by Mr. Samuel N. Baxter of Philadelphia, in April, 1905, at a nursery near Philadelphia. A search of the correspondence in the files of the United States Department of Agriculture corroborates this statement.

The specimen which was sent to the United States Department of Agriculture was referred to the Mycologist, Mrs. F. W. Patterson, for

[1] Weir, James R. Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. I. Phytopath. 4: 272. 1914.

examination, and pronounced "a Peridermium which causes what is called a pine-blister rust."

A search in February, 1917, in the pathological collections of the Bureau of Plant Industry failed to reveal the specimen. The letter from Dr. L. O. Howard, dated April 22, 1905, referring the specimen to Dr. A. F. Woods, has the notation on it, "White Pine, Peridermium on" in the handwriting of the Mycologist.

Since there is no reason to believe that the determination was incorrect, and since but a single species of Peridermium has ever been reported as causing a blister rust on white pine, this specimen was in all probablity *Peridermium strobi* Klebahn, the pine stage of *Cronartium ribicola* Fisher. This record, then, antedates the finding by Stewart of the Cronartium stage on currants at Geneva, New York, in 1906, and the hitherto earliest record in this country of the Peridermium stage on pine discovered in New York on June 8, 1909, and reported by Spaulding in 1909.

ROY G. PIERCE

Needle rust on Pinus resinosa. In June, 1916, the writer saw at Sharon Vermont, a very striking case of needle rust in a plantation of 10,000 trees of *Pinus resinosa* about 4½ feet in height. Up to about 3 feet the yellow spore bodies, although small, were so abundant on the 1915 needles as to be readily discernible 15 to 20 feet away, single needles bearing as many as 20 pustules. Hedgcock has identified the rust as belonging to the two species, *Coleosporium solidaginis* (Schw.) Thum. and *C. delicatulum* (Long) Hedgc. & Long. The alternate hosts were abundant in the plantation as well as beyond it. This seems to be a case of healthy trees being brought to that locality and infected by the fungi which were already present on the herbaceous hosts. Because of the abundance of the rust the writer thought it might be a serious matter to such small trees. A second visit made in September, however, showed that the needles were a healthy green color except for small dead spots where the rust pustules were produced. It is possible that the damage may later become more evident and this point will be determined.

PERLEY SPAULDING

Notes on the distribution of the bacterial disease of western wheat-grass.[1] Until recently the writer had not observed the bacterial disease of western

[1] O'Gara, P. J. A paper read before the meeting of the American Phytopathological Society, Columbus, Ohio, December 28, 1915.
Abstract published in Phytopathology **6**: 98–99. 1916.
Science n. s. **42**: 616–617. 1915.
Phytopath. **6**: 341–349. 1916.

wheat-grass, *Agropyron smithii* Rydb., caused by *Aplanobacter Agropyri* O'Gara, outside of two counties within the state of Utah. The disease has been recently found, however, occurring on western wheat-grass in three widely separated districts of Montana, namely, Lewis and Clark, Broadwater, and Deer Lodge counties.

As noted in previous papers, this disease is most characteristic in that the yellow bacterial ooze is found to cover the glumes of the inflorescence and appears also in droplets of considerable size on the outside of the glumes and on the sheaths. It also causes some dwarfing of the plants as well as a bending of the stem above the last internode. When the inflorescence is infected germinable seeds are not produced.

The fact that this disease has been found in widely separated districts would indicate that it may be found wherever western wheat-grass grows. The writer would appreciate a note from anyone finding this disease, as he is interested in establishing the extent of is distribution.

P. J. O'GARA

The occurrence of Colletotrichum solanicolum O'Gara on eggplant. A note concerning this species of Colletotrichum was published as an abstract in Phytopathology.[1] Later a description of the organism appeared in

FIG. 1 EGGPLANT WILTED BY COLLETOTRICHUM SOLANICOLUM O'GARA. ONLY ONE PLANT (JUST ABOVE THE CROSS) IN THE FIELD WAS FREE FROM THE DISEASE

Mycologia.[2] At the time of publishing my earlier notes there was some doubt as to the parasitism of this new species. Cultures were exchanged

[1] O'Gara, P. J. A disease of the underground stems of Irish potato caused by a new species of Colletotrichum. Phytopath. 4: 410-411. 1914.

[2] O'Gara, P. J. New species of Colletotrichum and Phoma. Mycologia 7: 38-41. 1915.

with Dr. J. J. Taubenhaus, then at the Delaware Agriculture College, who concluded that the organism is a species of Colletotrichum and that it has parasitic tendencies (oral communication).

During the summer of 1916 the writer had occasion to observe a field of eggplants in which fully ninety per cent of the plants showed wilt. At first it was supposed that the wilt was due to a Fusarium, noting only the general appearance of the field. A careful examination of the plants, however, indicated that Fusarium was not present but that the roots and stems were badly infected with the above-named organism. Interior portions of infected roots and stems were taken under sterile conditions and placed in culture tubes, where the organism fruited characteristically. No other organism appeared in the cultures where the proper precautions were taken. Even in mixed cultures, Fusarium did not appear.

When the organism was studied as a root and stem parasite of the potato, it was not suspected of being a serious wilt fungus, but in the case of the eggplant it has been found to produce a wilt disease similar to that produced by Fusarium. During the early growth of the plants no trouble was noted, but about the time some of the earlier fruits began to mature, the infected plants wilted. This condition shortly prevailed throughout the entire field, producing an almost total loss.

The field in which these eggplants were grown had supported a stand of potatoes the previous year and it was in this field of potatoes that the writer first found the above-named organism. From specimens collected in this field the original description was made.

This note is published in order to give notice of the economic character of this species of Colletotrichum.

P. J. O'GARA

Personals. Mr. K. E. Quantz, formerly assistant plant pathologist at the Virginia Experiment Station, has become plant pathologist to the Brazilian Government, with headquarters at Rio de Janeiro.

Mr. H. E. Thomas has resigned his position as instructor in plant pathology at the Virginia Polytechnic Institute to accept an appointment as assistant pathologist at the Federal Experiment Station at Mayaguez, Porto Rico.

Mr. Fred R. Jones, formerly a graduate student at the University of Wisconsin, has been appointed to the position of pathologist in charge of forage crop disease investigations, Bureau of Plant Industry, beginning April 14, 1917.

Mr. Gustav A. Meckstroth, a student at Pennsylvania State College, has accepted an appointment as scientific assistant in plant pathology, Office of Cotton, Truck and Forage Crop Disease Investigations, Department of Agriculture, beginning May 1, 1917.

LITERATURE ON PLANT DISEASES[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

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Arthur, Joseph Charles. Uredinales of Porto Rico based on collections by H. H. Whetzel and E. W. Olive. Mycologia 9: 55–104. F. 1917.

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Bisby, G. R. The short-cycled Uromyces of North America. (Abstract.) Phytopathology 7: 74. F. 1917.

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Buller, Arthur Henry Reginald. Black rust of wheat. Bul. Misc. Inform. Kew, 1917: 48. F. 1917.

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Burkholder, W. H. Bean diseases in New York State in 1916. (Abstract.) Phytopathology 7: 61. F. 1917.

Dry root rot, *Fusarium* sp.; blight, *Bacterium Phaseoli*.

Butler, Ormond Rourke. How to control the snapdragon rust. Florists' Ex. 43: 353. F. 17, 1917.

Dusting with sulphur.

Byars, Luther Parris. A nematode disease of the dasheen and its control by hot water treatment. (Abstract.) Phytopathology 7: 66. F. 1917.

—— Tylenchus tritici on wheat. Phytopathology 7: 56–57. F. 1917.

[1] Beginning with this number, it is intended that this list shall include all references to the literature of plant diseases, both American and foreign. All foreign articles published since January 1, 1917, which come to our attention, will be entered, so that the index may be ultimately considered complete from that date.

All authors are urged to cooperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

Carsner, Eubanks. Do the bacteria of angular leaf spot of cucumber overwinter on the seed? (Abstract.) Phytopathology 7: 61–62. F. 1917.

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——— Mycelium of the white pine blister rust. (Abstract.) Phytopathology 7: 77. F. 1917.
Cronartium ribicola.

——— Pycnial scars, an important diagnostic character for the white pine blister rust. (Abstract.) Phytopathology 7: 77. F. 1917.

Cromwell, Richard O. Fusarium-blight, or wilt disease, of the soybean. Jour. Agr. Research 8: 421–440, 1 fig., pl. 95. Mr. 12, 1917.
Literature cited, p. 438–439.
Fusarium tracheiphilum Smith.

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"Bordeaux has no effect on the rust of snapdragons, and will not control this disease."

Doubt, Sarah L. The response of plants to illuminating gas. Bot. Gaz. 63: 209–224. 6 fig. Mr. 1917.
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Durrell, W. L. Notes on curly dwarf symptoms on Irish potatoes. (Abstract.) Phytopathology 7: 71. F. 1917.

Edgerton, Claude Wilbur. A Gnomonia on eggplant. (Abstract.) Phytopathology 7: 78. F. 1917.
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——— Dissemination of the angular leafspot of cotton. Jour. Agr. Research 8: 457–475, fold. chart. Mr. 19, 1917.
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——— ———. A Peridermium belonging to Coleosporium terebinthinaceæ. (Abstract.) Phytopathology 7: 67. F. 1917.

——— - - -- Some new hosts for Coleosporium inconspicuum. (Abstract.) Phytopathology 7: 68 69. F. 1917.

——— ——— Some new hosts for Coleosporium solidaginis. (Abstract.) Phytopathology 7: 68. F. 1917.

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——— Investigations of the rotting of slash in Arkansas. U. S. Dept. Agr. Bul. 496, 14 p. 1917.

Oak slash:—*Stereum rameale, S. umbrinum, S. versiforme, S. fasciatum*
Shortleaf-pine slash:—*Polystictus abietinus, Lenzites sepiaria.*

None of the main fungi concerned in rotting either the oak or the pine slash in Arkansas produce heart rots in living trees.

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Botryorhiza Hippocratea Whetzel & Olive, sp. nov.; ***Endophyllum circumscriptum*** (Schw.) Whetzel & Olive, comb. nov.; ***Endophyllum Wedeliæ*** (Earle) Whetzel & Olive, comb. nov.; ***Endophyllum decoloratum*** (Schw.) Whetzel & Olive, comb. nov.; ***Endophyllum Stachytarphetæ*** (Henn.) Whetzel & Olive, comb. nov.; ***Endophylloides portoricensis*** Whetzel & Olive, sp. nov.

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THE CUPRAMMONIUM WASHES

Their Preparation, Biological Properties, and Application

O. Butler

With Plates III to X

A number of cuprammoniums have been recommended as fungicides, but, despite the fact that they form, or it would be more accurate to say, could be made to form, clear solutions and leave, on drying, inconspicuous spots on the foliage, no single one has sufficiently met the desiderata of practice to become generally employed. Practice demands that a fungicide be non-toxic to the sprayed plant at the strength at which it is most efficient and effective, but the cuprammoniums have the reputation of being unduly toxic and of not being as effective though more efficient than Bordeaux mixtures.

The toxicity of the cuprammoniums has been ascribed to soluble copper (Millardet[1] being notably a proponent of this view), to excess ammonia, and to ammonium sulphate. The prevailing opinion regarding the ineffectiveness of the cuprammoniums is based on their behavior relatively to Bordeaux mixtures which, in fact, is comparing solutions weak in copper with mixtures considerably stronger. Hawkins,[2] for instance, compared a cuprammonium containing 0.053 per cent copper with Bordeaux mixture containing 0.18 to 0.25 per cent copper. The question may, therefore, be asked, are any or is any one of the cuprammoniums worthy of being retained amongst the fungicides? In order to satisfactorily answer this question it will be necessary to study: (1) The composition and preparation of the different washes. (2) The relative toxicity of the different washes, and the conditions affecting the same.

[1] Millardet, A., and Gayon, U. Les divers procédés de traitement du mildiou par les composés cuivreux. Journ. Agr. prat. 1: 729. 1887.

[2] Hawkins, L. A. Grape-spraying experiments in Michigan in 1909. U. S. Dept. Agr. Circ. 65. 1909.

(3) The relative efficiency and effectiveness of the cuprammoniums. This we will now proceed to do.

I. CHEMISTRY AND METHODS OF PREPARATION OF THE CUPRAMMONIUMS

In the preparation of the cuprammonium washes met with in practice either ammonium hydroxid or ammonium carbonate are employed as the solvent, the solute being, in the case of the former solvent, either copper turnings, i.e., metallic copper, copper sulphate, the basic copper carbonate malachite ($CuCO_3$. $Cu(OH)_2$), or the basic copper carbonate of Burgundy mixture (2 $CuCO_3$. 3 $Cu(OH)_2$)[3] and in the case of the latter the copper salts mentioned, together with basic copper sulphate and cuprammonium sulphate ($CuSO_4$. 4 NH_3. H_2O). With the exception of the copper sulphate-ammonia wash which is a cuprammonium sulphate, all the other washes prepared with ammonium hydroxid produce cuprammoniums of very similar, if not identic composition and form a well characterized group; similarly the washes prepared with ammonium carbonate are also compositionally so nearly alike as to form a well defined group. The former are cuprammonium hydrates, the latter cuprammonium carbonates. Since cuprammonium sulphate was the first introduced of the cuprammonium washes and the cuprammonium hydrates were introduced prior to the cuprammonium carbonates, it will be possible both to retain a group distribution and consider the several fungicides in chronological order.

A. Cuprammonium sulphate washes

Copper sulphate and ammonia. The copper sulphate and ammonia wash (eau céleste) was the first introduced of the cuprammonium fungicides and still remains, taking the world at large, the best known. It is the most easily prepared and at the same time the most unstable, even when sufficient ammonium hydroxid is employed to give a clear solution. The fungicide was introduced by Audoynaud[4] in 1885, but the original formula which is as follows:

	part
Cupric sulphate	1
Ammonium hydroxid sp. gr. 90	0.769[5] by volume
Water to	100

[3] Bedford, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. 11: 86. 1910.

[4] Audoynaud, A. Le mildiou et les composés cupriques. Progrès agr. et vit. 1885.

[5] Equivalent of ammonium hydrate sp. gr. 92, 1 part by volume called for in the original formula.

proved injurious to vegetation and has suffered more or less marked modifications at the hands of subsequent writers as will be seen from a perusal of table 1.

TABLE 1

Strength of copper sulphate and ammonia wash and ratio cupric sulphate: ammonium hydroxid recommended by different authors

STRENGTH IN CUPRIC SULPHATE	RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM HYDROXID SP. GR. 0.90}}$
per cent	
0.26	1:1.50
0.50	1:0.76
0.50	1:1.06
0.50	1:1.70
0.50	1:1.14
1.00	1:0.73
1.00	1:1.06
1.00	1:1.46

The formulae given in table 1 do not necessarily represent the composition of the fungicide when put in service, however, for we find authors recommending that it be allowed to stand after being made for a few hours, half a day or even several days[6] so that the excess of ammonium hydroxid may pass off. Nor do the formulae permit, except in one instance, i.e., in the case of the wash containing 1 per cent cupric sulphate and a ratio cupric sulphate-ammonium hydroxid of 1:1.46, of the fungicide being applied as a perfectly clear solution which is essential if the copper is to be deposited on the sprayed foliage in the proper physical and chemical states. Millardet[7] long ago pointed out that Audoynaud's formula was in this respect defective and increased the amount of ammonium hydroxid so as to give a ratio of 1:1.53 instead of 1:0.769. The ratio cupric sulphate ammonium hydroxid required to give a clear 1 per cent solution (and Millardet's figures are correct) has to be greatly increased in order to prevent a precipitate forming when washes containing less than 1 per cent cupric sulphate are employed. The Duke of Bedford and Pickering[8] have shown that the amount of ammonium hydroxid required increases very considerably with the dilution and the data presented in table 2 confirms this view. But while the data given in the table show a very considerable increase in the amount of ammonium

[6] Viala, P. Les maladies de la vigne, ed. 3, p. 143. 1893.

[7] Millardet, A., and David, E. Essais comparatifs de divers procédés de traitement du mildiou. Compte-rendu Congrès nationale viticole, Bordeaux. Appendix, 60. 1886.

[8] Woburn Exp. Fruit Farm Rept. 11: 18. 1910.

TABLE 2

Amount of ammonium hydroxid required to give clear solutions of copper sulphate and ammonia of different strengths in cupric sulphate together with the corresponding ratios cupric sulphate: ammonium hydroxide and percentages of ammonia

STRENGTH IN CUPRIC SULPHATE	AMOUNT AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION[1]	RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM HYDROXID}}$	STRENGTH OF SOLUTION IN NH_3 INDICATIVE ONLY
per cent	*per cent*		*per cent*
1 0	1.5	1:1.5	0.386
0 5	1 0	1:2 0	0 255
0 2	0.8	1:4 0	0 204
0.1	0 7	1:7 0	0 179

[1]The data given under the heading amount of ammonium or ammonium carbonate, as the case may be, required to give a clear solution indicates the amount of the salt required to give a clear solution for a period of time of not less than two hours in a closed vessel.

hydroxid relative to the copper, it also brings out equally clearly that the absolute amount of ammonia[9] in solution decreases as the concentration of the copper is lowered. It may also be well to add that owing to the extreme volatility of ammonium hydroxid the figures given in the table will have to be increased at temperatures much above 20°C. and conversely may be somewhat decreased for temperatures below 20°C.

The copper sulphate and ammonia wash may be most conveniently prepared by adding the ammonium hydroxid required to a strong solution of copper sulphate and diluting immediately after the precipitate first formed has dissolved, though the wash may be satisfactorily prepared even when the relative dilution of the salts varies within wide limits. It is generally considered that the copper and ammonia wash (and the other cuprammoniums) are best prepared with soft water since the solutions are decomposed by hard water. The amount of copper precipitated when hard water is used is, however, entirely negligible in practice as may be judged from table 3.[10]

TABLE 3

Amount of copper precipitated from the copper sulphate and ammonia wash by waters of different degrees of hardness

SOURCE OF WATER	HARDNESS OF WATER HYDROTIMETRIC DEGREES	COPPER PRECIPITATED
		per cent
City of Bordeaux	26 0	0 00086
Gayon well	92 0	0 00406

[9]The term ammonia is used to denote NH_3 unless the context indicates the connotation NH_4OH

[10]Millardet, A., and Gayon, U. Les divers procédés de traitement du mildiou par les composés cuivreux. Journ. Agr. prat. 1: 732. 1887.

When ammonium hydroxid is slowly added to a strong solution of cupric sulphate a precipitate of basic cupric sulphate ($CuSO_4$. 2 $Cu(OH)_2$) is thrown down which dissolves in an excess of the reagent forming a deep blue solution, the copper being then in the form $CuSO_4$. 3 NH_3. 2 H_2O[11] or, according to the more commonly accepted view $CuSO_4$. 4 NH_3. H_2O a salt which decomposes readily on volatilization of ammonia or on dilution, the copper being precipitated as a basic sulphate. When the fungicide dries upon foliage the copper is deposited as a basic sulphate mixed with a little ammonium sulphate in the ratio of 1:0.35; in other words only a very small amount of the latter salt can be present even when a 1 per cent solution is sprayed on foliage as will be clearly seen from a study of the reactions involved which are as follows:[12]

(1) $3\ CuSO_4\ 5\ H_2O + 4\ NH_4\ OH = CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)_2SO_4 + 15\ H_2O.$

(2) $CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)_2SO_4 + \frac{8\ NH_4OH}{\text{in excess}} = 3\ (CuSO_4.\ 4\ NH_3.H_2O) + 9\ H_2O.$

(3) $3\ (CuSO_4.\ 4\ NH_3.H_2O) + H_2O = CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)SO_4 + 8\ NH_3$

B. Cuprammonium hydrate washes

A cuprammonium hydrate is formed when metallic copper, cupric oxide, cupric hydrate, malachite, or the basic cupric carbonate of Burgundy mixture are dissolved in ammonium hydroxid, and washes have been used in practice prepared from copper and all the copper salts mentioned with the exceptions of cupric oxid and hydrate. All the cuprammonium hydroxid washes decompose on dilution or on volatilization of ammonia with formation of cupric hydrate, the copper also being deposited in this form when the fungicides dry spontaneously on foliage. The cuprammonium hydrate washes are more stable than cuprammonium sulphate.

Copper and ammonia wash. The copper and ammonia wash, or Schweizer's reagent, was first introduced as a fungicide by Bellot des Minières in 1887[13] but despite the fact that the results he obtained are said to have been highly satisfactory it is practically unknown in the literature.

For the preparation of the copper and ammonia wash a very large amount of ammonium hydroxid is required and the copper must be acted

[11] Bedford, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. **11**: 17. 1910.

[12] Chester, F. D. The copper fungicides. Journ. Myc. **6**: 23. 1891.

[13] Bellot des Miniéres, H. Ammoniure de cuivre et parasites de la vigne. 1887.

on in the presence of air or traces of ammonium salts. Bellot des Minières employed the former method and prepared a stock solution which was diluted at time of use so as to contain 0.25 to 0.75 per cent metallic copper, i.e., the copper equivalent of a 1 to 3 per cent Bordeaux mixture. The stock solution was made as follows:

	parts
Copper turnings	1
Ammonia, sp. gr. 0.9	119 by volume

A stock solution prepared in the manner indicated can be diluted with water to 0.0317 per cent copper without a precipitate forming within a period of two hours. At this dilution, however, the solution contains 0.97 per cent ammonia which, as a glance at table 4 will show, is much higher than in the other cuprammoniums of equivalent strength in copper. The fact coupled with the difficulties incident to the preparation of the stock solution has seriously militated against the employment of the fungicide in practice, the advantages incident to its use, i.e., an adhesiveness equal to the copper sulphate and ammonia wash[14] and lesser injuriousness to the grape,[15] being not sufficiently compensatory to outweigh the drawbacks.

TABLE 4

Amount of ammonia required to give clear solutions of the cuprammonium washes when containing 0.0317 per cent metallic copper

NAME OF FUNGICIDE	AMOUNT NH_3 REQUIRED TO GIVE A CLEAR SOLUTION
	per cent
Copper-ammonia	0.9772
Copper sulphate-ammonia	0.179
Copper sulphate-ammonium carbonate	0.049
Malachite-ammonia	0.6821
Burgundy mixture-ammonia	0.360
Malachite-ammonium carbonate	0.047
Burgundy mixture-ammonium carbonate	0.038

Burgundy mixture and ammonia. This the earliest copper carbonate and ammonia wash employed in practice was introduced by Patrigeon in 1887[16] and consists simply in dissolving the basic copper carbonate of Burgundy mixture directly in the mother liquor by means of ammonium hydroxid. The wash is used to some extent in practice and

[14] Foëx, G. Cours complet de Viticulture ed. 4. 577.

[15] Foëx, G. The same, p. 578.

[16] Patrigeon, G. Nouveaux procédés de traitement du mildiou. Journ. agr. prat. 1: 882. 1887.

is known in the United States under the name of modified eau céleste. The formula originally proposed was as follows:

	parts
Copper sulphate	1
Sodium carbonate	1.5
Ammonia sp. gr. 0.9	0.769 by volume
Water to	100

Patrigeon's formula has not suffered any marked modifications at the hands of the various authors who mention it as will be seen from table 5.

TABLE 5

Strength of Burgundy mixture-ammonia and corresponding ratios copper sulphate sodium carbonate, and copper sulphate ammonium hydroxid recommended by different authors

STRENGTH IN CUPRIC SULPHATE	RATIO $\frac{\text{COPPER SULPHATE}}{\text{SODIUM CARBONATE}}$	RATIO $\frac{\text{COPPER SULPHATE}}{\text{AMMONIUM HYDROXID}}$
per cent		
0.25	1:1.20	1:0.83
0.30	1:1.25	1:0.83
0.50	1:1.25	1:0.83
0.50	1:1.25	1:1.04
1.0	1:1	1:0.73
1.1	1:1.25	1:0.78

The amount of ammonia called for in the various formulae for the preparation of modified eau céleste is never sufficient to give clear solutions. The basic carbonate of copper of Burgundy mixture, separated from the mother liquor by decantation is, however, readily soluble in ammonium hydroxid, as may be gathered from table 6.

TABLE 6

Amount of ammonium hydroxid required to give clear solutions of modified eau céleste

STRENGTH IN COPPER SULPHATE	AMOUNT OF AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*	*per cent*
1.00	4	1.029
0.50	3	0.772
0.25	2.5	0.642
0.10	1.4	0.360

Burgundy mixture dissolves in ammonium hydroxid, forming cuprammonium hydrate and is not a mixture as Chester[17] believed of cupram-

[17] Chester, F. D. Report of the Mycologist. Delaware Agr. Exp. Sta. Rept. 4: 68. 1891.

monium hydrate and carbonate. Modified eau céleste does not give rise to malachite on decomposing thus indicating absence of cuprammonium carbonate. When the wash dries upon sprayed foliage the copper is deposited as an hydrate, mixed with some sodium sulphate and bicarbonate unless the copper carbonate is separated from the mother liquor and levigated before being dissolved as is sometimes recommended.

Copper carbonate and ammonia. The copper carbonate and ammonia wash, or ammoniacal copper carbonate was introduced by Gastine[18] and in American fungicide literature is the cuprammonium most commonly met with.

Malachite dissolves in ammonium hydroxid forming a solution which, according to the Duke of Bedford and Pickering,[19] consists mainly of cuprammonium carbonate, while Chester[20] is of the opinion that both cuprammonium carbonate and hydrate are formed. As, however, the malachite ammonia wash does not deposit malachite on standing, I incline to the view that the copper is present solely as cuprammonium hydrate.

Malachite dissolves sparingly in ammonium hydroxid, the total amount entering into solution being less in a strong than a weak concentration of ammonia. Penny[21] for instance, found that a 42.68 per cent ammonium hydroxid dissolved, per gram weight of ammonia, 0.01329 grams of metallic copper while under similar conditions a 21.38 per cent solution dissolved 0.3132 grams and a 3.20 per cent solution 1.063 grams metallic copper respectively. These results, unfortunately for practice, can not be obtained by dissolving a given quantity of malachite in the suitable corresponding strength of ammonium hydroxid. In order to obtain the maximum solvent action it is necessary to use a very large excess of malachite, "even five fold or more" which introduces obvious difficulties that can not be turned except in a very empirical and unsatisfactory manner.

The malachite-ammonia wash was, as I have already indicated, proposed by Gastine whose formula was as follows:

	parts
Malachite	0.06
Ammonia sp. gr. 0.90	0.769
Water to	100

The above formula has been more or less modified at the hands of subsequent writers, as will be seen from table 7.

[18] Gastine, G. Emploi du carbonate ammoniacal de cuivre contre le peronospora. Prog. agr. et vit. **8**: 1887.

[19] Woburn Exp. Fruit Farm Rept. **11**: 21. 1910.

[20] The same, p. 68.

[21] Penny, C. L. The preparation of ammoniacal solution of copper carbonate. Delaware Agr. Exp. Sta. Bul. **22**: 5. 1893.

TABLE 7

Strength of malachite-ammonia wash and corresponding ratios malachite: ammonium hydroxid recommended by different authors

STRENGTH IN MALACHITE	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM HYDROXID}}$
per cent	
0.045	1:22.2
0.046	1:5.5
0.060	1:12.8
0.075	1:11.3
0.078	1:10
0.078	1:13.2
0.086	1:10
0.093	1:8.7
0.093	1:5.5
0.097	1:10
0.097	1:13.2
0.100	1:1.23
0.100	1:11
0.125	1:8
0.200	1:8

Neither in the original formula nor any of the subsequent ones that have come to my knowledge is the ratio malachite-ammonium hydroxid such as to insure complete dissolution of the copper salt. In order to dissolve the malachite completely, at least within a reasonable time, the ratio must be increased to 1:30. It is therefore clear that, as usually prepared, the wash either contains less copper than the formulae call for, or if the undissolved malachite is incorporated in the wash then the copper will be placed on the plants partly in the form of malachite and partly as a copper hydrate. In order to obviate these difficulties I have employed a stock solution prepared as follows:

	parts
Malachite	1
Ammonia sp. gr. 90	30 by volume
Water	20 by volume

In preparing the stock solution water must be used as otherwise the solution would prove unstable, decomposing with formation of cupric oxid. A stock solution containing as little as five parts water may be prepared and probably more water than the formula calls for could be used, though it will be perfectly obvious that there is no object in making a stock solution unduly dilute. In fact, *ceteris paribus* highly concentrated solutions are to be preferred.

The stock solution as above prepared is quite stable and may be diluted very considerably without decomposing, but contains more ammonia than

TABLE 8

Amount of ammonium hydroxid required to give clear solutions of malachite-ammonium hydroxid

STRENGTH IN TERMS OF COPPER SULPHATE	AMOUNT AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION	STRENGTH OF SOLUTION IN NH_3
per cent	*cc.*	*per cent*
1	26 5	6 82
0.5	13 25	3.41
0 25	6 62	1.70
0 10	2 65	0.682

modified eau céleste for equal percentages of copper (Cu) as may be seen by comparing table 8 with table 6. There is, therefore, no justification, since sodium sulphate and bicarbonate are not injurious at the concentrations at which the wash may be employed in practice, in the preference accorded the malachite-ammonia wash, as the copper occurs in the same form in both. The difference in favor of modified eau céleste is not only marked, however, in the formulae I have used, but is also favorable to the latter when we compare the formulae of authors. Taking the extremes met with we find the results shown in table 9 which are even more favorable to modified eau céleste than in the case of my formulae. And when we consider the cost of the unit copper employed (exclusive of labor) in modified eau céleste and malachite-ammonia we obtain a ratio of 1:3 which is so significant as to require no comment.

TABLE 9

Extreme percentages of ammonia met with in the formulae of authors for the preparation of modified eau céleste (A) and malachite-ammonia (B) respectively

STRENGTH OF SOLUTION IN TERMS OF COPPER SULPHATE	STRENGTH OF SOLUTION IN NH_3	
	A	B
per cent	*per cent*	*per cent*
0 25	0 051	0 564
	0 073	0 141
1 1	0 206	0 707
	0 294	2 828

C. Cuprammonium carbonate washes

Cuprammonium carbonate washes are formed when cupric sulphate, malachite, cuprammonium sulphate ($CuSO_4 \cdot 4\ NH_3 \cdot H_2O$) and the basic cupric carbonate of Burgundy mixture are dissolved in ammonium carbonate ($NH_4HCO_3 \cdot NH_4 \cdot NH_2CO_2$). The washes are very stable, the most stable of the cuprammonium fungicides, only decomposing slowly

on long standing with formation of malachite. When the washes dry on sprayed foliage the copper is deposited as a carbonate.

Cuprammonium sulphate-ammonium carbonate. This wash was introduced by the United States Department of Agriculture in 1890[22] under the name of mixture number 5 but has never been used to any extent in practice as it was not found to possess a lesser toxicity than other cuprammoniums and is not economical to prepare. The formula originally proposed was as follows:

	parts
Ammoniated copper sulphate (Cuprammonium sulphate)	0.21
Ammonium carbonate	0.12
Water to	100

Malachite-ammonium carbonate. This fungicide was introduced by Chester[23] in 1891 as a substitute for the malachite-ammonia wash, but despite manifest advantages has been but little used in practice. Chester's formula is as follows:

	parts
Malachite	0.052–0.058
Ammonium carbonate	0.27 –0.31
Water to	100

The original formula has been modified to some extent by subsequent writers as is shown in table 10.

TABLE 10

Strength of malachite-ammonium carbonate wash and corresponding ratios malachite: ammonium carbonate recommended by different authors

STRENGTH IN MALACHITE	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM CARBONATE}}$
per cent	
0.039	1:6
0.046	1:5.3
0.052	1:5.19
0.058	1:5.34
0.066	1:3.3
0.093	1:5.3

Malachite dissolves fairly readily in ammonium carbonate, carbon dioxid and a little ammonia being evolved during the reaction, but the reaction is not sufficiently rapid nor the conditions under which it takes place such as to permit the preparation of the fungicide as required. A stock solution is necessary and may be conveniently prepared as follows:

[22] United States Department of Agriculture Rept. 1890: 402.

[23] Delaware Agr. Exp. Sta. Rept. 4: 71. 1891.

	parts
Malachite	1
Ammonium carbonate (hard)[24]	3
Water	20

Place the malachite in a suitable non-metal vessel, add the ammonium carbonate in small pieces, and then the water. Warm gently and as soon as effervescence begins remove from flame and stir. Let stand a few minutes, place back on flame and continue as before until on warming gently no further effervescence takes place. The vessel should then be closed and set aside until the malachite has completely dissolved, should it not already have done so. The stock solution prepared as above will withstand marked dilution without a further addition of ammonium carbonate being required at least within the range of concentration in copper that I have used, as will be seen from table 11.

TABLE 11

Amount of ammonium carbonate (hard) required to give clear solutions of malachite-ammonium carbonate

STRENGTH IN MALACHITE	AMOUNT AMMONIUM CARBONATE REQUIRED TO GIVE A CLEAR SOLUTION	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM CARBONATE}}$	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*		*per cent*
0.50	1.5	1:3	0.476
0.20	0.6	1:3	0.190
0.10	0.3	1:3	0.095
0.05	0.15	1:3	0.047

Copper sulphate and ammonium carbonate. The copper sulphate and ammonium carbonate wash known in American literature as Johnson's mixture has been but little used in practice and is but rarely mentioned by writers on the fungicides. The wash was first described in 1891 by Johnson[25] who proposed the following formula for its preparation:

	parts
Copper sulphate	0.1
Ammonium carbonate (hard)	0.20
or Ammonium carbonate (soft)	0.25
Water to	100

[24] Ammonium carbonate decomposes on exposure to air and in preparing the stock solution the amount of the salt used will have to be increased unless it is in perfectly hard translucent plates. When completely decomposed ammonium carbonate occurs as an opaque powder and when in this condition the amount called for in the formula should be doubled.

[25] Johnson, S. W. Note by the Director. Connecticut Agr. Exp. Sta. Rept. 1890: 113. 1891.

Johnson's mixture is, next to the copper sulphate and ammonia wash (eau céleste), the most easily prepared of all the cuprammoniums. When ammonium carbonate is added to a strong solution of cupric sulphate a precipitate is first formed accompanied by effervescence due to the liberation of carbon dioxid which on a further addition of ammonium carbonate promptly and completely dissolves even in the cold.

Johnson's mixture forms very stable solutions and, as a glance at table 12 will show, is for equivalent of copper compositionally identical with malachite-ammonium carbonate.

TABLE 12

Amount of ammonium carbonate hard and soft required to give clear solutions of Johnson's mixture and corresponding ratios cupric sulphate: ammonium carbonate

STRENGTH IN CUPRIC SULPHATE	AMOUNT AMMONIUM CARBONATE REQUIRED TO GIVE A CLEAR SOLUTION		RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM CARBONATE}}$[1]	STRENGTH OF SOLUTION IN NH_3[1]
	Hard	Soft		
per cent	*per cent*	*per cent*		*per cent*
1.00	1.56	2.40	1:1.56	0.495
0.50	0.78	1.20	1:1.56	0.247
0.20	0.31	0.48	1:1.56	0.099
0.10	0.15	0.24	1:1.56	0.049

[1] Refers to solutions prepared with undecomposed ammonium carbonate.

Burgundy mixture-ammonium carbonate. Burgundy mixture may be readily dissolved in ammonium carbonate yielding a cuprammonium very similar to those obtained with malachite, copper sulphate, or cuprammonium sulphate as will be seen by a glance at table 13.

TABLE 13

Amount of ammonium carbonate required to give clear solution of Burgundy mixture-ammonium carbonate and corresponding ratios Burgundy mixture: ammonium carbonate

STRENGTH IN COPPER SULPHATE	AMOUNT AMMONIUM CARBONATE (HARD) REQUIRED TO GIVE A CLEAR SOLUTION	RATIO $\frac{\text{BURGUNDY MIXTURE}}{\text{AMMONIUM CARBONATE}}$	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*		*per cent*
1.00	1.20	1:1.20	0.380
0.50	0.60	1:1.20	0.190
0.25	0.30	1:1.20	0.095
0.10	0.12	1:1.20	0.038

The data given in table 13 are for a Burgundy mixture in which the ratio copper sulphate sodium carbonate (crys.) was 1:1.84, and the precipitate was separated from the mother liquor before dissolving in the

ammonium carbonate. In practice, however, the precipitate would not need to be separated from the mother liquor, since the salts therein contained are not injurious[26] at the concentrations at which the wash may be employed in practice.

As a result of our study of the properties and preparation of the cuprammonium washes we may conclude:

1. The cuprammonium carbonate washes are the most stable and for strengths in metallic copper of 0.14 per cent or less require less ammonia to give clear solutions than the washes prepared with ammonium hydroxid.

2. The copper salts dissolved in ammonium carbonate yield washes in which the active principle, i.e., the copper is in the form of a carbonate.

3. Metallic copper, malachite, and the basic carbonate of Burgundy mixture form cuprammonium hydrates when dissolved in ammonium hydroxid modified eau céleste requiring per equivalent of copper the least amount of solvent.

4. Cupric sulphate forms with ammonium hydroxid a cuprammonium sulphate which is the least stable of the cuprammonium fungicides, though less ammonia is required to give a clear solution than in the case of the cuprammonium hydrates.

II. RELATIVE TOXICITY OF THE CUPRAMMONIUM WASHES

The cuprammonium washes may be toxic to the sprayed plant: (1) Between the time of application and time of drying; (2) after drying owing to dissolution of the contained copper on weathering; or (3) the injury produced, if any, may be the result of the additive effect of 1 and 2. There are no other possibilities.

A. Effect of the cuprammoniums on plants between the time of application and the time of drying

It will be at once evident that if the cuprammoniums made with ammonium hydroxid owe their toxicity to the presence of ammonia, the toxic action, owing to the rapid dissipation of ammonia in the interim between time of application when its concentration will be highest and time of desiccation when its concentration will be zero, must and can only take place during the drying of the spray. Ammonium carbonate is less volatile than ammonium hydroxid, but nevertheless decomposes readily on exposure to air and has vanished when the washes of which it is a component have dried upon the foliage, hence injury due to the presence of ammonia in ammonium carbonate can also only be produced in the

[26] See p. 257.

TABLE 14

Effect of quick and slow drying on the toxicity of the cuprammonium washes[1] expressed in per cent of injury

VARIETY USED	HOW DRIED	FUNGICIDE USED AND STRENGTH AT WHICH EMPLOYED															
		Copper sulphate-ammonia				Johnson's mixture				Malachite-ammonia				Malachite-ammonium carbonate			
		Per cent Cu				Per cent Cu				Per cent Cu				Per cent Cu			
		0.254	0.127	0.0635	0.0317	0.254	0.127	0.0635	0 0317	0.2874	0.1437	0.0718	0.0359	0.2874	0.1437	0.0718	0.0359
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coleus var. Golden Bedder	Quickly	15	8	0	0	5	2	0	0	32	17	0	0	3	0	0	0
	Slowly	28	19	9	3	15	9	4	2	83	43	17	4	65	34	14	2
Tomato var. Bonny best	Quickly	2	1	0	0	5	0	0	0	40	16	5	0	3	0	0	0
	Slowly	11	6	0	0	39	9	0	0	100	72	30	4	72	55	28	16
Bean var. Dwarf horticultural	Quickly	23	9	7	3	37	27	19	5	40	26	13	4	24	11	10	4
	Slowly	27	13	10	5	46	30	20	8	60	45	24	5	29	16	10	7
Pelargonium varieties	Quickly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slowly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] The data presented in the tables of this chapter are the mean values of four duplicate experiments though sometimes more and in a few instances only three have been performed. The plants dried quickly were always dry within two hours while those dried slowly were placed in moist chambers, taken out at the end of six hours and allowed to dry spontaneously. The cuprammoniums were applied as perfectly clear solutions and the reader desirous of ascertaining the exact formula employed in any particular case may readily do so by turning to the appropriate table in the previous chapter.

interim between time of application and time of drying. If the toxic action is due, on the other hand, to soluble copper or ammonium sulphate whenever this salt occurs, then the injury resulting may be produced during the time of drying and also at such subsequent times as the fungicide is wetted by meteoric water. The rate at which the cuprammoniums dry upon sprayed foliage will, it may therefore be presumed, have a very marked effect on the degree of injury produced. And in fact experimental evidence fully confirms the presumption as is shown in table 14 and plates III and IV.

The data presented in the above table show how significant was Audoynaud's recommendation that the copper sulphate and ammonia wash be applied during dry, warm weather and fully justifies Bourcart's[37] statement that scorching "is especially to be feared when eau céleste is applied during moist weather and, consequently, when it dries slowly on the leaves;" but that on the other hand "when the spray is applied during the hot days of summer, this fault disappears entirely, and eau celeste possesses nothing but advantages." The data presented in the table show conclusively that slow drying is much more injurious than quick drying, the difference being very striking indeed in the case of malachite-ammonia and malachite-ammonium carbonate containing 0.28 per cent and 0.14 per cent copper.

While the data presented in table 14 show that whenever a cuprammonium is toxic it is invariably more injurious when dried slowly irrespective of the plant sprayed, it does not give us any very definite information regarding the cause of the deleteriousness of these washes. The data do not support conclusively either the view that ammonia is the toxic agent, or the view that the toxicity is due to soluble copper since were but one of these components the sole cause of the injury produced a certain proportionality would exist differing only in degree in different plants between a given strength of the toxic substance and the resulting injury, but a consideration of table 14 shows that no such relation exists in either case. On the other hand, the data clearly show that ammonium sulphate does not possess any markedly injurious properties. Neither are the malachite washes shown to be less injurious than those made from cupric sulphate and ammonia, a fact worthy of serious consideration in view of the general abandonment of the latter on account of supposed greater toxicity.

But since no individual component of the cuprammonium washes is apparently *per se* the cause of the toxic action produced in the interval between the application of the fungicide and its desiccation it will be

[37] Bourcart, E. Les maladies des plants, 376. Paris. 1910.

necessary for us to study their behavior when applied separately in order to interpret the data given in table 14, and to the action of soluble copper we may well devote attention first.

The only suitable copper salt to use in studying the effect of soluble copper when applied in the form of a spray is cupric sulphate and since the acid radicals of the copper salts are not in themselves injurious[28] the toxicity of cupric sulphate may be considered as due to the copper. Soluble copper is, as is well known, extremely toxic to vegetation and we would, therefore, expect that the injury produced by an application of cupric sulphate would be the greater the slower the spray dried on the foliage, and the data presented in table 15, fully confirm this expectation, and the illustration shown in plate V is no less emphatic.

TABLE 15

Effect of quick and slow drying on the toxicity of cupric sulphate expressed in per cent of injury

VARIETY USED	HOW DRIED	MILLIGRAMS PER CENT COPPER (CU.)[1]									
		508	254	127	63	31	15	7.9	3.9	1.9	0.99
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coleus	Quickly		14	7	3	2	0				
	Slowly		37	18	8	5	4				
Tomato	Quickly	40	18	10	8	4	0	0			
	Slowly	90	67	55	32	16	10	0			
Cauliflower var. Snowball	Quickly			42	39	29	18	15	7	5	0
	Slowly			58	53	35	31	28	15	14	2
Bean	Quickly				50	29	19	0	0	0	
	Slowly				80	56	37	20	8	0	
Oxalis bowei	Quickly			33	19	13	5				
	Slowly			60	48	29	20				
Pelargonium Martha Washington var.	Quickly	20	11	3	0						
	Slowly	60	26	13	5						

[1] The heading, used also in plate viii, means that the percentage strength of the solution is given in terms of milligrams instead of fractions of a gram.

The data presented in table 15 and shown graphically in plate VIII indicate that the tomato, Oxalis, bean and cauliflower are much more sensitive to soluble copper than either the Coleus or the Pelargonium. The tomato, bean, Oxalis and cauliflower respond in a somewhat similar manner to soluble copper, the curves for quick and slow drying being

[28] Bedord, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. 11: 1910.

Clark, J. F. On the toxic properties of some copper compounds with special reference to bordeaux mixture. Bot. Gaz. 33: 39. 1902.

Hawkins, L. A. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals towards fungus spores. Physiological Researches 1: 87. 1913–16.

very nearly parallel at the higher concentrations. In the case of the cauliflower which is very sensitive to soluble copper the curves are nearly proximate, while in the case of the tomato, which is much more tolerant, they are distant. The curves for the Oxalis and bean are very similar in character, and resemble those of the tomato, though the bean is more sensitive to soluble copper than the Oxalis and the Oxalis less resistant than the tomato. In the most highly resistant plants studied, the Pelargonium and Coleus, the curves show marked similarity, the toxicity on slow drying increasing a little more rapidly in the former than in the latter.

It will also be noticed that while in the more resistant plants (Coleus, Pelargonium) the toxicity is practically proportional to the concentration in the less resistant plants proportionality ceases to exist as soon as the threshold of toxicity is approached, the decrease being much more rapid than the data for the higher concentrations would lead one to anticipate. The practical importance of these facts will escape no one.

TABLE 16

Effect of slow and quick drying on the toxicity of ammonium hydroxid sp. gr. .90, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION IN NH_3								
		10.29 per cent	5.14 per cent	4.11 per cent	6.57 per cent	2.05 per cent	1.28 per cent	1.02 per cent	0.51 per cent	0.25 per cent
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Coleus	Quickly				18			10	7	0
	Slowly				76			25	8	2
Tomato	Quickly			50		29		20	12	5
	Slowly			70		50		46	34	22
Cauliflower	Quickly	60	22		10		0		0	0
	Slowly	75	33		21		5		3	0
Bean	Quickly			23		16		2		
	Slowly			47		35		10		

The toxicity of ammonium hydroxid has been considered high by all writers who have held the tenet that the toxicity of the copper and ammonia washes was due to the ammonium hydroxid though Millardet poured, he tells us, strong ammonium hydroxid on the foliage of the grape without producing injury, a result strongly contradicting the prevailing view, and the data presented in table 16 are not on analysis favorable to it.

A glance at the table reveals that like the cuprammoniums and cupric sulphate ammonium hydroxid even though highly volatile is much more toxic when dried slowly and the illustration given in plate VI shows very strikingly what marked differences may be obtained. The data also show that, of all the plants used, the tomato is the most sensitive

to ammonium hydroxid while the Coleus, bean and cauliflower follow in increasing order of resistance. Now if ammonium hydroxid is primarily the cause of the toxicity of the cuprammoniums of which it is a component part the relative resistance of the plants when sprayed with the copper sulphate and ammonia wash or the malachite-ammonia wash should remain unchanged. In reality we find that when the former is employed the sensitiveness of the plants is in decreasing order as follows: Tomato, bean, Coleus;[20] and when the latter is used that it is as follows: Tomato, Coleus, bean. The evidence is therefore clear that ammonium hydroxid is not the primary cause of the toxicity of the cuprammoniums.

The toxicity of ammonium hydroxid is of course due to the ammonia (NH_3) it contains and it would therefore be expected that ammonium carbonate which has been used in the preparation of cuprammonium washes would also prove injurious since while less volatile and alkaline than the former it nevertheless decomposes rapidly on exposure to the air with liberation of ammonia and carbon dioxid. And in fact experimentation shows ammonium carbonate to be much more toxic than usually supposed as will appear from a consideration of table 17.

TABLE 17

Effect of slow and quick drying on the toxicity of ammonium carbonate, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION IN NH_3			
		1.90 per cent	0.95 per cent	0.47 per cent	0.23 per cent
		per cent	*per cent*	*per cent*	*per cent*
Coleus	Quickly	40	2	0	0
	Slowly	100	81	0	0
Tomato	Quickly	40	15	5	0
	Slowly	100	70	50	20
Cauliflower	Quickly	75	29	10	7
	Slowly	92	42	13	6
Bean	Quickly		1	0	0
	Slowly		8	0	0

The data presented in table 17 and more strikingly illustrated in the graphs (plates IX and X) show that within the limits of toxicity ammonium carbonate is much more injurious per equivalent of ammonia than ammonium hydroxid and it is rather difficult to understand how it could ever have come to be considered less noxious than the former. As would be expected from the nature of the salt, the toxicity of ammonium car-

[20] The cauliflower was not used in the experiments on the toxicity of the cuprammoniums.

bonate when dried slowly is much more marked than when it is dried quickly. The illustration (plate VII, fig. 1), will show what striking effects may be obtained.

The data show that of all the plants studied the tomato is the most sensitive to ammonium carbonate, the susceptibility of the other plants being in order of increasing resistance as follows: Coleus, cauliflower, bean. Similarly in the case of ammonium hydroxid, the relative susceptibility of the plants is in increasing order as follows: Tomato, Coleus, bean, cauliflower. The relative toxicity of ammonium hydroxid and ammonium carbonate is clearly shown in plates IX and X. An inspection of the graphs on these plates shows that the toxicity of ammonium carbonate, whether dried quickly or slowly, increases much more rapidly with the concentration than does the toxicity of ammonium hydroxid, the curves for the former indicating proportionality between concentration and toxicity while those for the latter resemble those given by copper sulphate. A consideration of plate IX shows that ammonium carbonate is more toxic to the bean and cauliflower irrespective of the rate at which dried than ammonium hydroxid. From plate X, on the other hand, we gather that in the case of the tomato ammonium carbonate is less toxic between 0 and 0.95 per cent ammonia than ammonium hydroxid when dried quickly, but is invariably more toxic when dried slowly; in the case of the Coleus ammonium carbonate dried quickly is less toxic between 0 and 1.02 per cent ammonia than ammonium hydroxid and, when dried slowly, less toxic between 0 and 0.51 per cent.

Ammonium sulphate has been considered by certain writers as the toxic agent of the cuprammoniums in which it occurs and the copper sulphate and ammonia wash has suffered relegation in consequence of this view. Pearson,[30] however, at an early date impugned this belief for he found that the mother liquor of a strong copper sulphate ammonia wash caused no injury to the grape vine, the strawberry and "various other vegetables." A glance at table 18 will show that ammonium sulphate can not possibly be the cause of the toxicity of the cuprammoniums in which it occurs, since the amount of ammonium sulphate formed could not exceed 0.53 gram for every gram of cupric sulphate employed. But if ammonium sulphate can not be the cause of the toxicity of the copper sulphate and ammonia wash or Johnson's mixture, the data presented in the table show that it possesses a peculiarity to which we may well devote a moment's attention.

[30] Pearson, A. N. *In* Report on the experiments made in 1888 in the treatment of the downy mildew and blackrot of the grape vine. U. S. Dept. Agr., Sec. Veg. Path. Bul. 10: 18. 1889.

TABLE 18

Effect of slow and quick drying on the toxicity of ammonium sulphate, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION			
		4 per cent	2 per cent	1 per cent	0.5 per cent
		per cent	*per cent*	*per cent*	*per cent*
Coleus	Quickly	91	66	24	0
	Slowly	24	19	0	0
Bean	Quickly	63	28	16	0
	Slowly	25	4	1	0
Tomato	Quickly	73	34	8	0
	Slowly	50	22	6	0

TABLE 19

Effects on tomato, Coleus and bean of the slowly dried cuprammoniums and their components at the strengths at which they occur in the washes, expressed in per cent of injury

FUNGICIDE USED	AMOUNT OF COPPER IN SOLUTION	TOMATO				COLEUS				BEAN			
		Cuprammonium	Cupric sulphate = soluble copper	Ammonia	Ammonium sulphate	Cuprammonium	Cupric sulphate = soluble copper	Ammonia	Ammonium sulphate	Cuprammonium	Cupric sulphate = soluble copper	Ammonia	Ammonium sulphate
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Copper sulphate and ammonia	0.254	11	67	27	0	28	37	3	0	27	100	0	0
	0.127	6	55	22	0	19	18	1	0	13	100	0	0
	0.063	0	32	15	0	9	8	0	0	10	80	0	0
	0.031	0	16	13	0	3	5	0	0	5	56	0	0
Johnson's mixture	0.254	39	67	34	0	15	37	0	0	46	100	0	0
	0.127	9	55	10	0	9	18	0	0	30	100	0	0
	0.063	0	32	0	0	4	8	0	0	20	80	0	0
	0.031	0	16	0	0	2	5	0	0	8	56	0	0
Malachite-Ammonia	0.287	100	67	91		83	37	100		60	100	51	
	0.143	72	55	64		43	18	100		45	100	45	
	0.071	34	32	50		17	8	49		24	80	28	
	0.035	4	16	38		4	5	14		5	56	0	
Malachite-Ammonium carbonate	0.287	72	67	50		65	37	39		29	100	0	
	0.143	55	55	18		34	18	0		16	100	0	
	0.071	28	32	0		14	8	0		10	80	0	
	0.035	16	16	0		2	5	0		7	56	0	

Ammonium sulphate is more toxic when dried quickly than when dried slowly, behaving in this respect exactly the reverse of the other components of the cuprammoniums all of which are much more toxic when dried slowly. The injury produced by quick drying may be very marked and the illustration, plate VII, fig. 2, will add but emphasis to the striking figures in the table. The explanation of this peculiarity is that ammonium sulphate is slightly hygroscopic and when dried quickly withdraws water from the leaf which wilts and dies, but that when dried slowly equilibrium between the cells of the epidermis and the solution is reached by the time drying begins.

The plant most seriously injured by quick drying is the Coleus, then follows the bean, the tomato being but little more seriously affected by quick drying than by slow drying, the data for 1 per cent solutions being in this respect particularly instructive. It would appear therefore that the cuticles of the Coleus and bean are not readily permeated by ammonium sulphate while that of the tomato is easily penetrated which facts are of considerable interest since slow drying which properly measures the toxicity of the salt shows that the most readily penetrated leaf is also the most susceptible.

Having concluded our study of the components of the cuprammoniums we are in a position to determine the nature of their rôle in the toxicity of these washes. In order to simplify our study I have placed *en regard* in table 19 the percentage injuries produced on the one hand by the several cuprammoniums and on the other the injury that would have followed the use of the components at the corresponding strengths at which they occur in the washes, the data being in all cases for slow drying.

The data presented in the table confirm in large measure the view of Millardet that soluble copper is the cause of the injury produced by the cuprammoniums though it would also appear that ammonia becomes toxic when its concentration exceeds an amount that may or may not be considerably in excess of that normally tolerated by the plant concerned. For instance in the case of the Coleus, malachite-ammonium carbonate containing 0.287 per cent copper, is more toxic than the equivalent strength of soluble copper or ammonia whence it must be concluded that the injury is due to combined action of the two; and it would appear from the behavior of this wash at other strengths that the presence of ammonia has in all cases even when itself not apparently toxic, increased the toxicity of the copper. The malachite-ammonium carbonate wash also shows evidence of an additive effect except at the lowest concentration used. On the other hand, Johnson's mixture shows that the toxicity of soluble copper is reduced by its presence despite the fact that the concentration of ammonia is very nearly the same as in malachite-ammo-

nium carbonate; but in the case of the copper sulphate and ammonia wash the evidence is conflicting. When we come to consider the tomato we find that the presence of ammonia reduces the toxicity of soluble copper in the case of Johnson's mixture and the copper sulphate and ammonia wash, but that in the malachite-ammonia wash its toxicity is increased except at the lowest concentration used. In the case of malachite-ammonium carbonate we have the toxicity of soluble copper increased at the highest concentration (0.287 per cent copper) not changed at 0.143 per cent copper and 0.035 per cent copper and reduced at 0.071 per cent copper.

Finally in the case of the bean we find that the presence of ammonia has in all cases reduced the toxicity of soluble copper.

The evidence is therefore in favor of the view that the presence of ammonia is beneficial and not injurious as too commonly supposed though it should be noted that while this beneficent action of ammonia is sufficiently general to be considered established and is independent of the rate at which the cuprammoniums decompose, the data nevertheless clearly show that cuprammonium sulphate, the most unstable of the washes, is also, all things considered, less injurious than cuprammonium hydrate and carbonates which are very stable. Soluble copper must therefore be the major cause of the toxicity of the cuprammoniums in the interim between application and desiccation.

B. Effect of the cuprammoniums after they have dried upon the plant and are subject to the action of the weather

After the cuprammoniums have dried upon the plant there will be present on the leaf only a copper carbonate or hydrate in the case of the washes prepared from malachite, but in the case of those prepared with cupric sulphate (copper sulphate and ammonia wash, Johnson's mixture) ammonium sulphate not in excess of one-half of the cupric sulphate employed will also be present, and in the case of the washes prepared from Burgundy mixture we will have besides the copper salt both sodium bicarbonate and sodium sulphate present, the former to the extent of 0.41 gram for every gram of cupric sulphate taken, the latter to the extent of 0.56 gram for every gram of cupric sulphate used.[31] In two instances, therefore, the dissolution of the copper in meteoric waters is not affected by the presence of a foreign substance, in two instances the foreign substances (sodium sulphate and bicarbonate) are neither toxic

[31] The calculations are based on the formula (vide Bedford, Duke of, and Pickering, S. U. loc. cit., p. 86). $5\ CuSO_4\ 5\ H_2O + 8\ Na_2CO_3 + 10\ H_2O = 2\ CuCO_3,\ 3\ Cu(OH)_2 + 6\ NaHCO_3 + 5\ Na_2SO_4 + 105\ H_2O$.

at the strengths at which they are found in the washes or solvents of the copper salt, and in two instances the action of meteoric waters is heightened by the presence of ammonium sulphate which is a solvent of insoluble copper salts but non-toxic itself at the strength at which it occurs in the washes. It is, therefore, clear that the toxicity of the cuprammonium washes after they have dried upon the leaf can only be due to the presence of soluble copper. Whence it will only be necessary, in order to determine the magnitude of this toxicity, to ascertain the degree of solubility of the copper in the several cuprammoniums as compared with that of a soluble copper salt, and, as already indicated, cupric sulphate is the only common inorganic salt of copper that satisfies the conditions under the experimental methods employed. It is desirable in testing the relative solubility of cupric sulphate and of the copper salt of the dried cuprammoniums that an organism sensitive to soluble copper be employed as indicator. The conidia of *Plasmopara viticola* are, as is well known, extremely sensitive to soluble copper[32] and are admirably adapted to this purpose and were employed in obtaining the data presented in table 20. The data were obtained by spraying microscopic slides after the manner described by Reddick and Wallace[33] with the solutions to be tested, allowing them to dry spontaneously at room temperature and putting in service not earlier than twenty-four hours after the fungicides had dried. In making a test the spores were washed with distilled water into a beaker from leaves just freshly gathered. Small drops of water with spores in suspension were taken from the beaker, and placed on the slides which were then incubated at or near the optimum temperature for indirect germination.[34] Simultaneously a witness was always prepared so that the vitality of the spores could be properly judged and no experiment was considered in which the germination in the witness proved low. Finally it should be noted that the data given in table 20 are the mean of five experiments except in the case of the malachite-ammonia wash with which only two tests were made.

The data show that the presence of ammonium sulphate increases the toxicity of a wash, the unit copper in copper sulphate and ammonium carbonate being more toxic than the unit copper in malachite-ammonium

[32] Millardet, A. and Gayon, U. Traitement du mildiou par le mélange de sulfate de cuivre et de chaux. Journ. agr. prat. 2: 709. 1885.

Wuthrich, E. Ueber die Einwirkung von Metallsalzen und Säuren auf die Keimfahigkeit der Sporen einiger des Verbreitetsten parasitischen Pilze unserer Kulturpflanzen. Zeit. Pflanzenkr. 2: 16-31, 81-94. 1892.

[33] Reddick, D. and Wallace, E. On a laboratory method of determining the fungicidal value of a spray mixture or solution. Science n. s. 31: 798. 1910.

[34] Melhus, I. E. Germination and infection with the fungus of the late blight of potato. Wisconsin Agr. Exp. Sta. Tech. Bul. 37: 36. 1915.

carbonate. It will be also noticed that the highest toxic value for the unit copper occurs in the copper sulphate-ammonia wash, though on account of the presence of ammonium sulphate it is impossible to tell to what extent this high value is due to the presence of this solvent, to what extent to the basic cupric sulphate. Judging from the increased toxicity of cupric carbonate in the presence of ammonium sulphate it would seem that the high toxic value of the unit copper in basic cupric sulphate is due to the presence of ammonium sulphate. However this may be, the table shows conclusively that the unit copper in the cuprammonium carbonate washes has a lower toxic value than in a cuprammonium hydrate or sulphate[35] wash.

TABLE 20

Relative toxicity of the cuprammonium washes and cupric sulphate to the spores of Plasmopara viticola

FUNGICIDE USED	LETHAL STRENGTH	SOLUBILITY OF COPPER
	per cent Cu	*Relative numbers*
Malachite-ammonia	0.0033	58.13
Malachite-ammonium carbonate	0.0057	33.33
Copper sulphate-ammonia	0.0027	70.42
Copper sulphate-ammonium carbonate	0.0035	54.34
Cupric sulphate	0.0019	100.00

Knowing the toxicity of cupric sulphate to any given higher plant it may be calculated readily from the data given in table 21 at what strength the cuprammoniums would have to be used in order to prove uninjurious after they had dried on the foliage and are wetted by meteoric water. Taking the plants used in the experiments with cupric sulphate[36] we arrive at the results shown in table 21, the data for the cupric sulphate being obtained from table 15, either directly or by extrapolation.

The data presented in the table show the strength at which the several cuprammoniums could be applied to foliage without producing injury after drying due to dissolution of the copper in meteoric waters. The question that we must now consider is whether these strengths are superior to or smaller than those at which injury occurs between time of application and time of drying. If inferior then the maximum strength at which the cuprammoniums can be used will be governed by the solubility of the copper after drying; if superior, then the degree of injury

[35] There is no reason for supposing that modified eau céleste or Burgundy mixture-ammonium carbonate would possess a toxicity materially different from their prototypes mentioned in the table.

[36] See p. 251.

produced while drying will limit the strength at which these fungicides may be used safely. The data presented in table 22 will permit us to answer the question raised.

The data show that in the case of all four fungicides the degree of tolerance of the dried fungicides must limit the strength at which they can be used.

TABLE 21

Calculated tolerance of plants to dried cuprammoniums

PLANT EMPLOYED	CUPRIC SULPHATE DRIED SLOWLY NON-TOXIC	MAXIMUM STRENGTH AT WHICH CUPRAMMONIUMS COULD BE USED			
		Malachite-ammonia	Malachite-ammonium carbonate	Copper sulphate-ammonia	Copper sulphate-ammonium carbonate
	per cent Cu	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*
Coleus	0 0075	0 0119	0 0225	0 0106	0 0138
Tomato	0 0075	0 0119	0 0225	0 0106	0 0138
Cauliflower	0 0007	0 00120	0 0021	0 00099	0 001 28
Pelargonium	0 0317	0 0545	0 0951	0 0450	0 0583
Bean	0 0019	0 0032	0 0057	0 0026	0 0034
Oxalis	0 0079	0 01358	0 0237	0 0112	0 0145

TABLE 22

Amount of copper tolerated in the several cuprammoniums during and after drying

FUNGICIDE USED	COLEUS		TOMATO		BEAN	
	Copper tolerated		Copper tolerated		Copper tolerated	
	During slow drying	After drying	During slow drying	After drying	During slow drying	After drying
	per cent Cu	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*
Malachite-ammonia	0 0298	0 01358	0 0327	0 01358	0 0287	0 0032
Malachite-ammonium carbonate	0 0375	0 0237	0 0287	0 0237	0 0183	0 0057
Copper sulphate-ammonia	0 0228	0 0112	0 0635	0 0112	0 0152	0 0026
Copper sulphate-ammonium carbonate	0 0254	0 0145	0 0635	0 0145	0 0228	0 0034

C. Injury by additive effect

As we have seen the cuprammoniums may produce injury during drying and after drying due to dissolution of the copper in meteoric waters. When the injuries produced by these sources is sufficiently distant, their mutual effects remain distinct, but when they occur nearly simultaneously an injury greater than that due to the sum of the effects

of the injury produced during drying and after drying is to be anticipated since as Schander[37] and Barker and Gimingham[38] have pointed out soluble copper is more injurious to recently than to remotely injured leaves. The data presented in table 22 show clearly that if injury is produced during drying, injury will also follow wetting with meteoric water. The converse is not, however, necessarily true, since the rate of drying markedly affects, as we have seen, the degree of tolerance of copper.

III. PRACTICAL CONSIDERATIONS

If we consider the strength at which the cuprammoniums are or have been used in practice, we will find that these fungicides are, or have been, as will be seen from table 23, applied at a strength in copper ranging from 7.2 to 47 times the lethal concentration for *Plasmopara viticola.*

TABLE 23

Strength at which the cuprammoniums have been most commonly employed in practice and lethal concentration of the same for Plasmopara viticola

FUNGICIDE USED	A	B	RATIO $\frac{A}{B}$
	Strength at which applied	Lethal concentration	
	per cent Cu	*per cent Cu*	
Malachite-ammonia	0.0493	0.0033	1:14.9
Malachite-ammonium carbonate	0.0264	0.0057	1:4.63
Copper sulphate-ammonia	0.0270	0.0027	1:47.03
Copper sulphate-ammonium carbonate	0.0254	0.0035	1:7.25

Now since the cuprammoniums may all be considered equally adhesive, as regards resistance to mechanical shock, because they form precipitates composed of particles of nearly like size, the concentration at which they are applied should bear a definite relation either to their efficiency or effectiveness.[39] But an inspection of table 23 will immediately show that no relation exists between lethal concentration and strength of application whether we take as our criterion efficiency or effectiveness. The

[37] Schander, R. Über die physiologische Wirkung der Kupfervitriol Kalkbrühe. Landw. Jahrb. **33**: —. 1904.

[38] Barker, B. T. P. and Gimingham, C. T. The action of Bordeaux mixture on plants. Ann. Appl. Biology **1**: 11 *et seq.* 1914.

[39] The efficiency of a fungicide depends both on the solubility of the copper and its toxicity when in solution. Usually, though not necessarily always, solubility is a measure of toxicity and conversely. Effectiveness depends on the power of a fungicide to withstand weathering, that is, to possess adhesive properties, while at the same time yielding sufficient soluble copper to give protection from a specific organism or organisms.

formulae in use should therefore be amended so as to give a numerical relation between lethal concentration and strength of application.

Let us first of all determine the factor required to give maximum effectiveness.

Since we have been unable to assign a value to this factor from the data presented in table 24, it will be necessary for us to determine it indirectly and this we can do from our knowledge of Bordeaux mixture. According to a recent French enquiry[40] a 2 per cent Bordeaux mixture is necessary to give adequate protection from *Plasmopara viticola* in years favorable to the development of this parasite though in years when infestation is not severe 1 per cent mixtures meet more or less satisfactorily the requirements of practice. In the United States, on the other hand, both *Plasmopara viticola* and *Phytophthora infestans* may be satisfactorily held in check by 1 per cent Bordeaux mixtures though 1.25 per cent mixtures are also commonly employed. We may therefore in all propriety take a 1 per cent Bordeaux mixture for our standard of comparison.

Now Bordeaux mixture 1:1 is toxic to both *Plasmopara viticola* and *Phytophthora infestans*[41] at 0.0039 per cent copper which gives us a factor of 64 as the requirement of practice for adequate protection. Accepting this factor of 64 for Bordeaux mixture the factors necessary to apply to the cuprammoniums, due regard being taken of the relative efficiency of the unit copper, in order to obtain satisfactory protection would then be as indicated in table 24.

TABLE 24

Factors by which the lethal concentrations of the several cuprammoniums must be multiplied in order to obtain adequate protection

FUNGICIDE USED	LETHAL CONCENTRATION	FACTOR
	per cent Cu	
Bordeaux mixture 1:1	0.0039	64
Malachite-ammonia	0.0033	53.7
Malachite-ammonium carbonate	0.0057	93.4
Copper sulphate-ammonia	0.0027	44.1
Copper sulphate-ammonium carbonate	0.0035	56.9

From the data given in table 24 one can readily determine the strength at which the cuprammoniums should be used in practice in order to obtain a protection substantially equivalent to that given by Bordeaux mixture, and when the data so obtained are compared with the strengths

[40] Capus, J. Les traitements du mildiou. Rev. de vit. **44**: 302. 1916.

[41] Wisconsin Agr. Exp. Sta. Tech. Bul. **37**: 36. 1915.

employed in practice, as in the following table, we find that the calculated strengths are from four to twenty times greater than those that have been actually used except in the case of the copper sulphate and ammonia wash when the agreement is close. But since the cuprammoniums have not afforded, at the strengths used in practice, protection commensurate with Bordeaux mixture and we are now in a position to understand why they have not, the formulae employed should be emended so as to approach the calculated values indicated. But the actual strengths employed will be determined by the tolerance of the plant sprayed to the fungicide in the interim between application and desiccation, and to the solubility of the dried wash in meteoric waters. We will first of all, consider the latter case.

The dried cuprammoniums must not, of course, yield on being wetted more soluble copper than the sprayed plant will stand. Let us accept

TABLE 25

Strengths at which the cuprammoniums are used in practice and strengths at which they should be employed in order to give protection equivalent to Bordeaux mixture

FUNGICIDE USED	STRENGTH USED IN PRACTICE	STRENGTH EQUIVALENT TO 1 PER CENT BORDEAUX MIXTURE
	per cent Cu	*per cent Cu*
Malachite-ammonia	0.0493	0.1772
Malachite-ammonium carbonate	0.0264	0.5323
Copper sulphate-ammonia	0.1270	0.1190
Copper sulphate-ammonium carbonate	0.0254	0.1991

for the sake of concreteness, the value for the tomato 0.0075 per cent copper. This percentage of metallic copper is yielded by a 0.045 per cent copper sulphate and ammonia wash under laboratory conditions but the tolerance of the tomato under field conditions may be safely placed at 0.125 per cent, since rains even of moderate intensity will carry away appreciable amounts of the fungicide. And since in the other cuprammoniums, as we have seen, the copper is less soluble than in the copper sulphate and ammonia wash, we may safely use them at the same relative concentration which would then give us the following as the permissible strengths at which they can be applied without injury from soluble copper resulting to a plant tolerating 0.0075 per cent soluble copper. As will appear from a consideration of table 26, the calculated values for soluble copper tolerated are lower than those obtaining in practice for the malachite-ammonia and copper sulphate-ammonia washes and higher in the case of the two cuprammonium carbonate washes.

The data presented in table 26 further show that it is impossible to apply the cuprammoniums at strengths equivalent in effectiveness to 1 per cent Bordeaux mixture when the plants sprayed will not tolerate more than 0.0075 per cent soluble copper since in order to obtain equivalence the plant sprayed would have to tolerate 0.0158 per cent soluble copper.

We have admitted that, under the conditions of practice plants will tolerate cuprammoniums 2.8 times stronger than tolerated under critical conditions. It remains now to be seen whether cuprammoniums of this strength can be applied without injury resulting during drying. The data presented in table 27 show that in the case of the tomato under the conditions of quick drying, all the cuprammoniums except the malachite-ammonia wash can be used at the required concentration, but that under the conditions of slow drying malachite-ammonia and malachite-ammonium carbonate, the latter particularly, are toxic at a lower concentration than that demanded. In the case of the Coleus we find quick

TABLE 26

Strengths at which the cuprammoniums may be used without an injurious amount of soluble copper forming on wetting with meteoric water, a plant resistant to 0.0175 per cent soluble copper being presupposed

FUNGICIDE USED	STRENGTH TOLERATED	STRENGTH USED IN PRACTICE
	per cent Cu	*per cent Cu*
Malachite-ammonia	0.0386	0.0493
Malachite-ammonium carbonate	0.0668	0.0264
Copper sulphate-ammonia	0.0317	0.127
Copper sulphate-ammonium carbonate	0.0410	0.0254

drying permits the use in every case of stronger solutions than tolerated in the dried wash, while the reverse is the case if slow drying is permitted. In the case of the bean the plant will tolerate stronger drying than dried washes. In the case of the tomato and Coleus the washes can all be used at 11.7 times their toxic concentration to *Plasmopara viticola* when dried quickly but in the case of the bean they can be applied at only 3.3 times their lethal concentration with safety, no matter how slowly or quickly the washes are dried. It seems, therefore, clear that the cuprammoniums can not be considered as effective as Bordeaux mixture for the control of parasitic organisms which do not require a concentration in soluble copper greater than that yielded by the latter. We have now to consider the cuprammoniums from the point of view of efficiency.

The unit copper in the cuprammoniums has generally been considered more efficient than the unit copper in Bordeaux mixture, and this opinion is undoubtedly well grounded when the cuprammoniums which are practically neutral as soon as dry are compared with alkaline Bordeaux mix-

tures in which the copper is without action during the time required to reach neutrality; but when compared with neutralized or neutral Bordeaux mixture at the lethal strengths to *Plasmopara viticola* the difference in favor of the cuprammoniums is indeed small. In the most efficient wash (copper sulphate and ammonia) the unit copper has a value only 1.44 times that of Bordeaux mixture and in the least efficient (malachite-ammonium carbonate) it is 1.46 times less active. But in fungi resistant to copper the unit copper in the cuprammoniums may be manifold that of Bordeaux mixture, the highest values being given by the copper sulphate and ammonia wash. For instance in the case of the uredospores of *Puccinia Antirrhini* the efficiency of the unit copper in the copper sulphate-ammonia wash is very much greater not to say infinitely greater than the unit copper in Bordeaux mixture since it would appear that the latter is non toxic at all concentrations.[42]

TABLE 27

Amount of copper tolerated in the cuprammonium washes during drying by the tomato, Coleus and bean

FUNGICIDE USED	TOMATO			COLEUS			BEAN		
	Strength of dried wash tolerated	Strength tolerated		Strength of dried wash tolerated	Strength tolerated		Strength of dried wash tolerated	Strength tolerated	
		Wash dried quickly	Wash dried slowly		Wash dried quickly	Wash dried slowly		Wash dried quickly	Wash dried slowly
	per cent Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu
Malachite-ammonia....	0.0386	0.0359	0.0327	0.0386	0.0718	0.0298	0.0089	0.0301	0.0287
Malachite-ammonium carbonate.....	0.0668	0.1437	0.0287	0.0668	0.1437	0.0315	0.0154	0.0229	0.0183
Copper sulphate-ammonia.....	0.0317	0.0635	0.0635	0.0317	0.0635	0.0228	0.0073	0.0203	0.0152
Copper sulphate-ammonium carbonate...	0.0410	0.127	0.0635	0.0410	0.0635	0.0254	0.0101	0.0279	0.0228

It is therefore clear that in the control of parasitic endophytes the spores of which are highly resistant to soluble copper, the cuprammoniums may be of very considerable value provided the plant to be sprayed will withstand the concentration demanded. But except in those cases where Bordeaux mixture is non toxic to the parasite from which protection is sought it does not seem to me that the cuprammoniums as a class or any one cuprammonium in particular possess merits sufficient to warrant

[42] Doran, W. L. Controlling snapdragon rust. Value of copper and sulphur. Florists' Exchange **43**: 501. 1917.

their employment in practice, especially since the quality of inconspicuousness can be obtained quite readily with Bordeaux mixtures 1: alkalinity Bordeaux mixture 1: alkalinity is no more conspicuous than the copper sulphate and ammonia wash when the same amount of copper is applied per square meter in both cases but since the latter usually wets the foliage better than the former it appears to be less highly colored, due to the fact that the copper is spread over a larger surface. Whenever Bordeaux mixture 1: alkalinity does not wet the foliage sufficiently to offer the proper degree of inconspicuousness, the wetting power of the mixture may be increased by the addition of an infusion of quillaia (i.e., saponin) or a small amount of casein. It is therefore not necessary to resort to a cuprammonium when an inconspicuous copper fungicide is required.

SUMMARY

1. The cuprammoniums met with in practice belong chemically in one or the other of the following groups: (1) cuprammonium sulphate; (2) cuprammonium hydrate, and (3) cuprammonium carbonate.

2. When decomposed by drying cuprammonium sulphate deposits the copper as a basic sulphate, cuprammonium hydrate yields a copper hydrate, and cuprammonium carbonate a copper carbonate.

3. Cuprammonium sulphate is very unstable; cuprammonium hydrate and carbonate very stable.

4. The cuprammoniums are more toxic when slowly than when quickly dried.

5. The toxicity of the cuprammoniums during drying and on weathering is due to soluble copper.

6. The copper sulphate and ammonia wash and Johnson's mixture are less injurious than the malachite washes.

7. The cuprammonium washes are more efficient and effective than Bordeaux mixture when large amounts of soluble copper are required to give protection.

8. The cuprammonium washes are less effective than Bordeaux mixture when small amounts of soluble copper suffice to give protection but with the exception of the malachite-ammonium carbonate wash are slightly more efficient.

9. The relative efficiency of the unit copper in the cuprammoniums is in decreasing order as follows: Copper sulphate-ammonia, malachite-ammonia, copper sulphate-ammonium carbonate, malachite-ammonium carbonate.

10. The cuprammoniums may be used at 11.7 times their lethal con-

centration for *Plasmopara viticola* on plants not affected by 0.0075 per cent soluble copper.

11. The cuprammoniums are of limited practical applicability and should not be used in lieu of Bordeaux mixture whenever the latter yields sufficient soluble copper to give protection.

NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION
DURHAM, NEW HAMPSHIRE

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DESCRIPTION OF PLATES

PLATE III

Effect of the malachite-ammonium carbonate wash containing 0.28 per cent Cu on the tomato var. Bonny best. Plants on the left dried quickly, plants on the right dried slowly. Photograph taken twenty-four hours after the fungicide was applied.

PLATE IV

Effect of the malachite-ammonia wash containing 0.28 per cent Cu on the tomato var. Bonny best. Plants on the left dried quickly, plants on the right dried slowly. Photograph taken twenty-four hours after wash was applied.

PLATE V

Tomato var. Bonny best photographed twenty-four hours after being sprayed with 1 per cent cupric sulphate. Plants on the left dried quickly, plants on the right dried slowly. The plants dried quickly showed slight scorching of young leaflets at the time of making the photograph but the injury was not sufficiently marked to show in the plate; the plants dried slowly were, on the other hand, very seriously injured all the leaves being withered and flaccid.

PLATE VI

Effect of ammonium hydroxid containing 4.11 per cent ammonia on the tomato var. Bonny best. Plants showing no apparent injury dried quickly, withered plants dried slowly. Photograph taken twenty-four hours after treatment.

PLATE VII

FIG. 1. Effect of ammonium carbonate containing 0.95 per cent ammonia on the Coleus var. Golden bedder. Plant on the right dried quickly, plant on the left dried slowly. The photograph was taken forty-eight hours after the salt was sprayed on the plants.

Fig. 2. Effect of a 2 per cent solution of ammonium sulphate on the bean var. Dwarf horticultural. Plant on the left dried slowly, plant on the right dried quickly. Photograph taken forty-eight hours after treatment.

PLATE VIII

Graphs showing the relative toxicity of quickly and slowly dried solutions of cupric sulphate to the Coleus, tomato, Oxalis, Perlargonium, bean and cauliflower

PLATE IX

Graphs showing relative toxicity of quickly and slowly dried solutions of ammonium hydroxid and ammonium carbonate to the bean and cauliflower.

PLATE X

Graphs showing relative toxicity of quickly and slowly dried solutions of ammonium hydroxid and ammonium carbonate to the tomato and Coleus

O. B. PHOTO

BUTLER: THE CUPRAMMONIUM WASHES

O. B. PHOTO

BUTLER: THE CUPRAMMONIUM WASHES

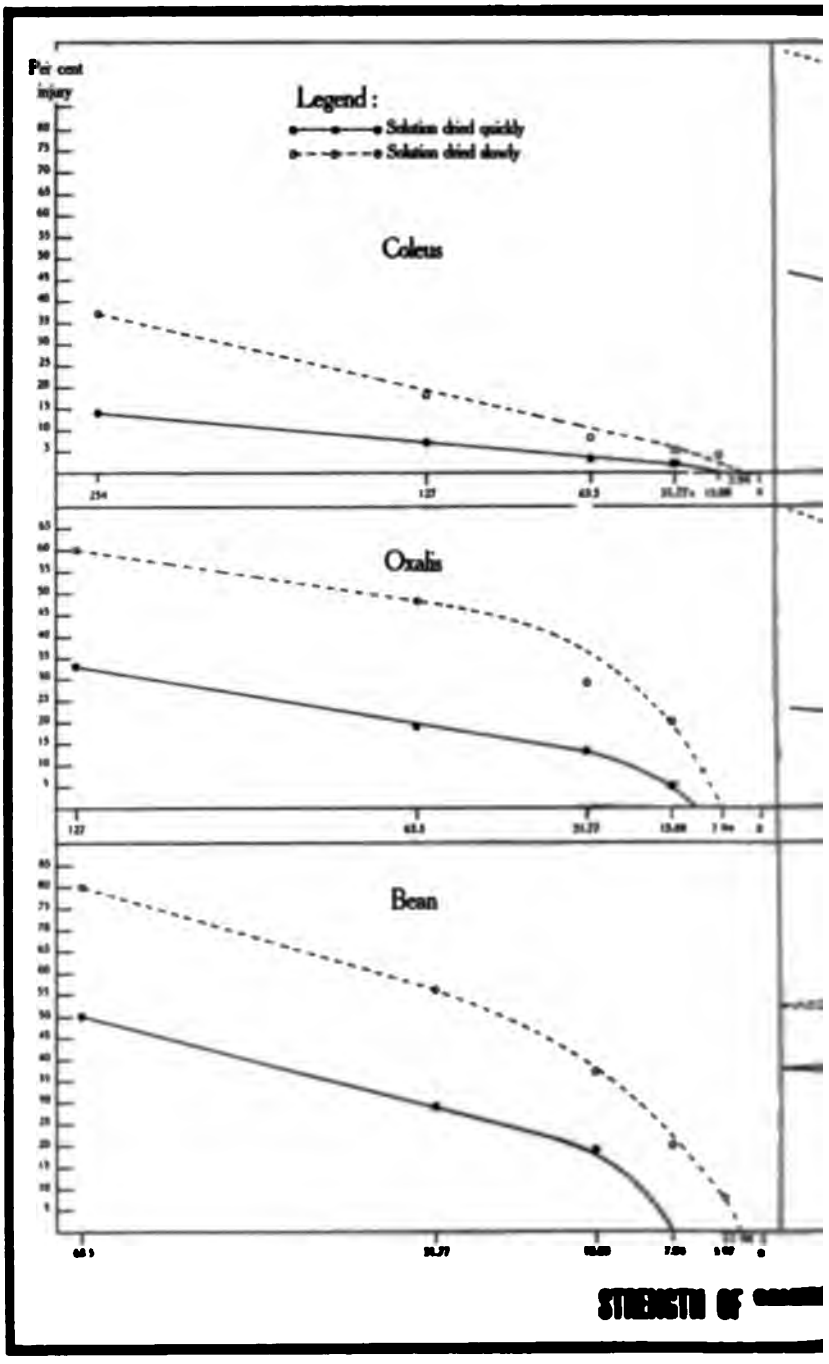
O. B PHOTO

BUTLER: THE CUPRAMMONIUM WASHES

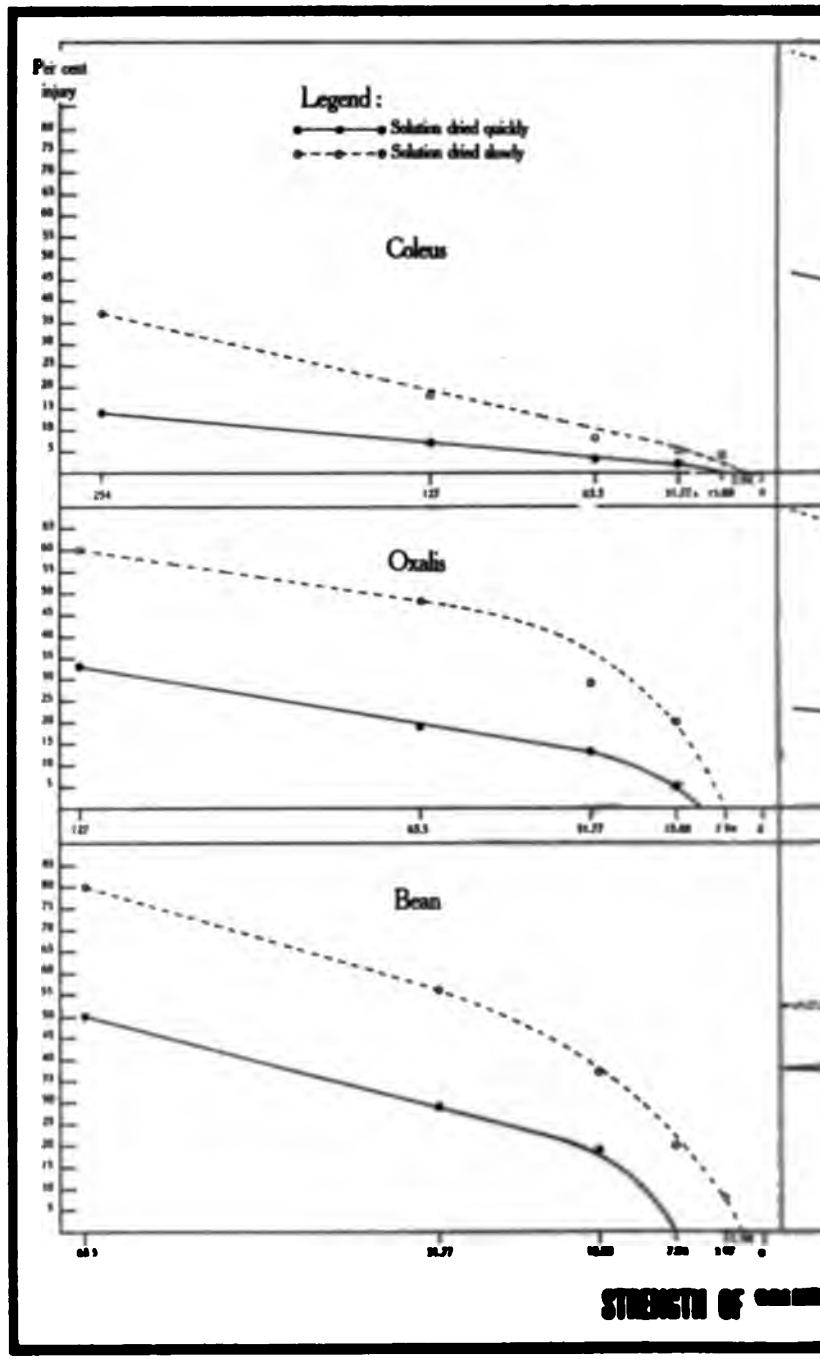
1

O. B. 2 PHOTO

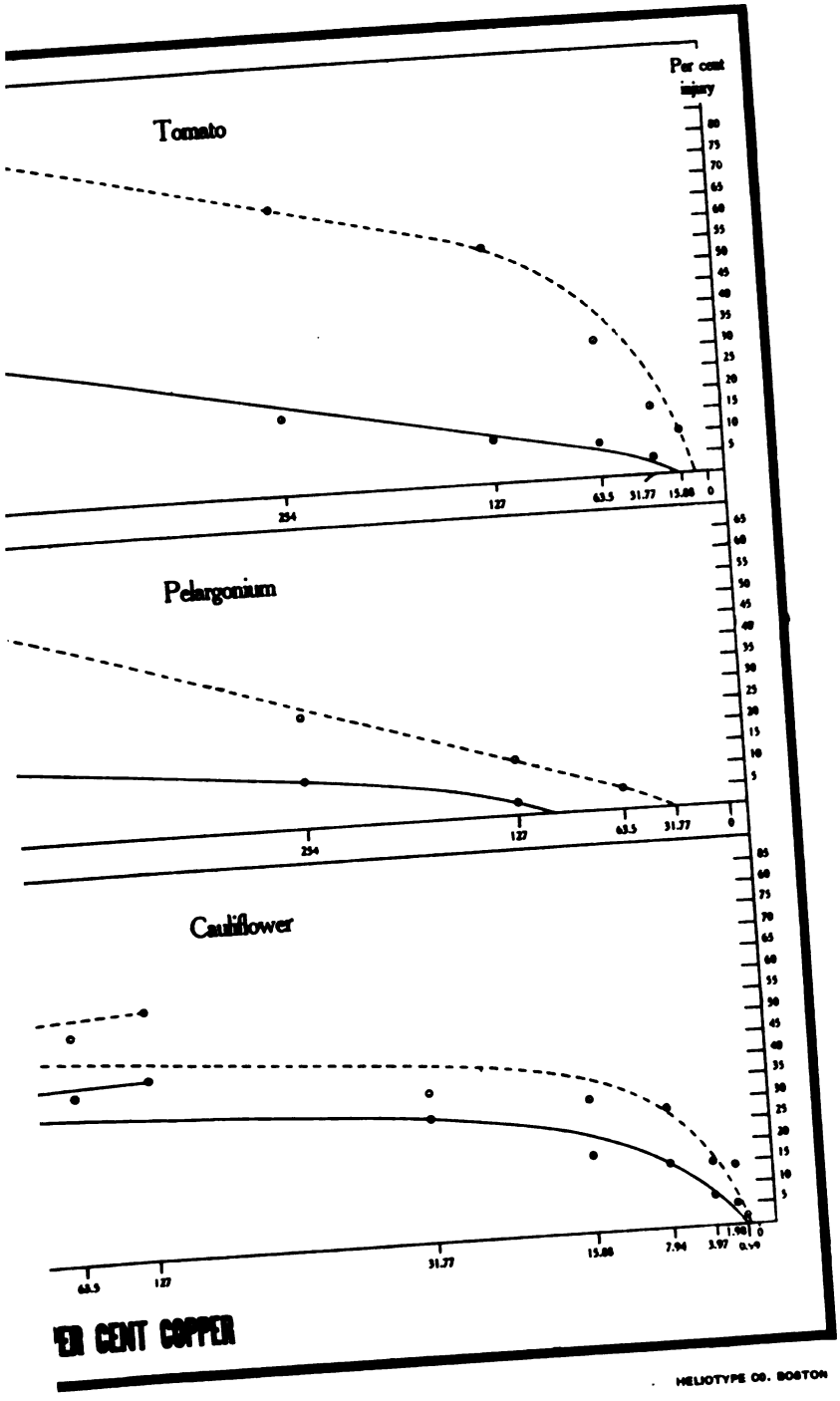
BUTLER: THE CUPRAMMONIUM WASHES


Per cent injury
Legend:
Solution dried quickly
Solution dried slowly
Coleus
Oxalis
Bean
STRENGTH OF

O. B. del.

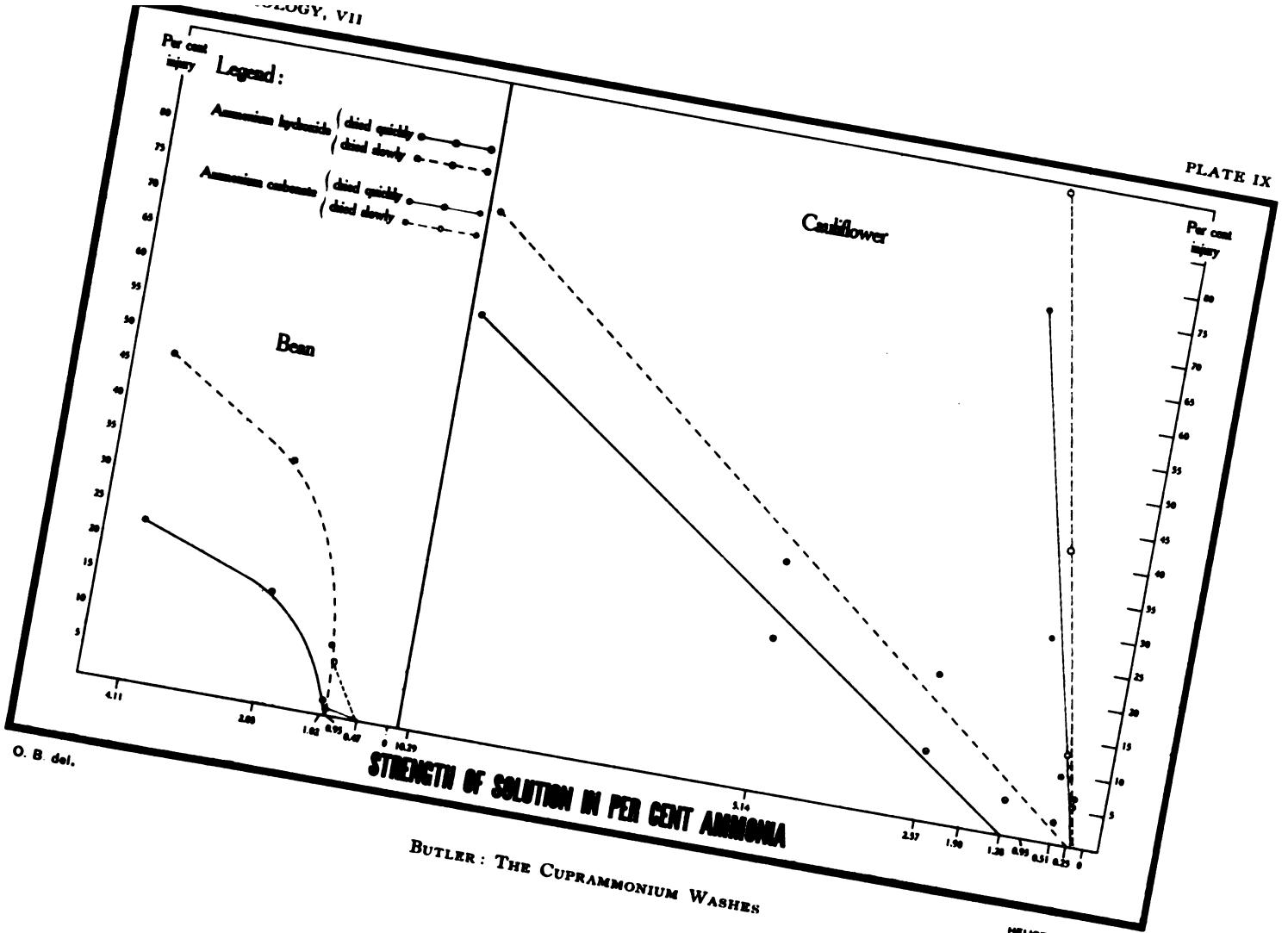

Per cent injury
Legend :
Solution dried quickly
Solution dried slowly
Coleus
Oxalis
Bean

O. B. del.



HELIOTYPE CO. BOSTON

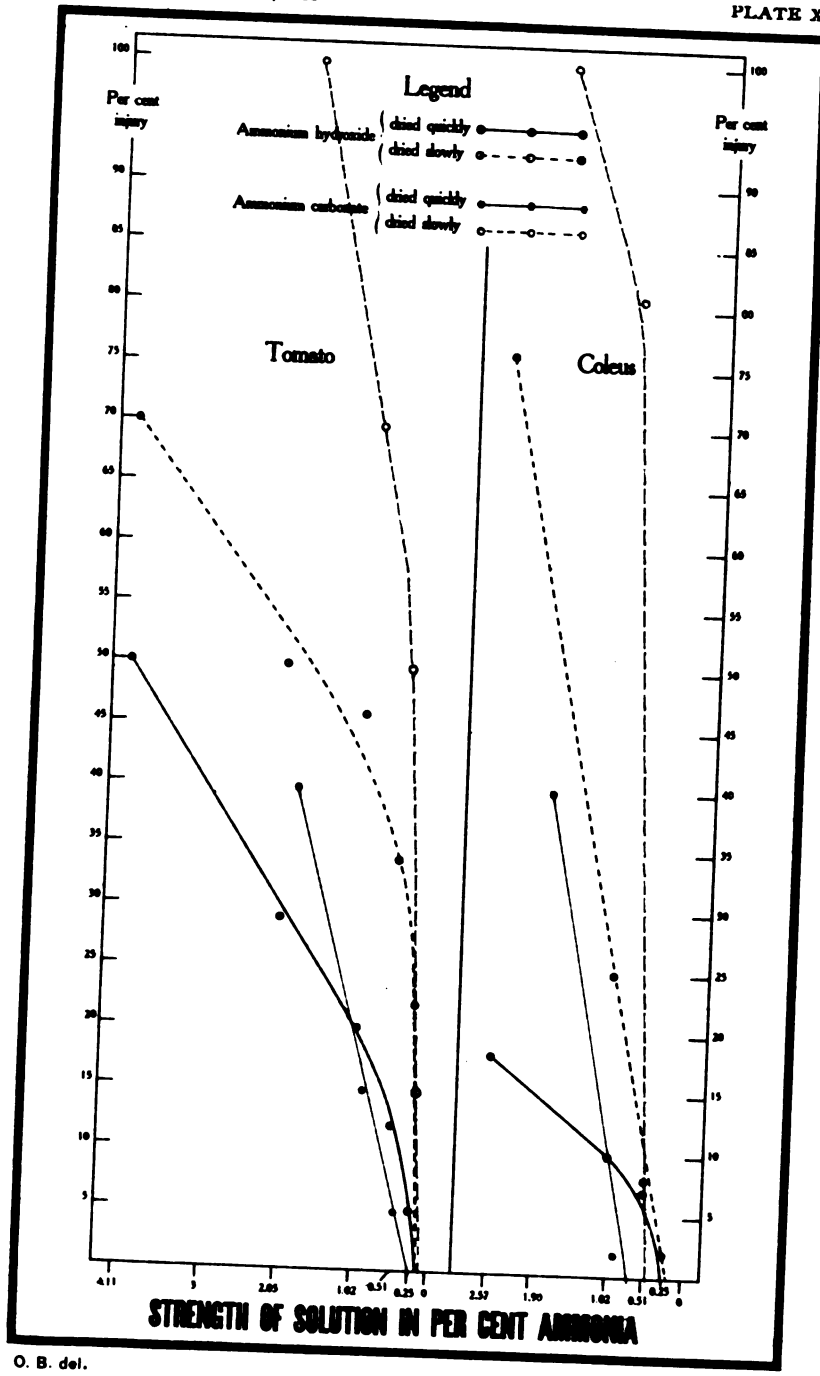
ASHES



O. B. del.

HELIOTYPE CO. BOSTON

PLATE X



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BACILLUS MORULANS N. SP.

A Bacterial Organism Found Associated with Curly Top of the Sugar Beet

P. A. Boncquet

With Seven Figures in the Text

The writer, in conjunction with Prof. Ralph E. Smith, has been investigating a peculiar and fundamentally important plant disease, the so-called curly top of the sugar beet and related plants. Two joint publications[1] have already been made upon this subject in which there has been briefly announced the discovery of a bacterial organism found constantly associated with the disease in the tissues of affected plants. It has been stated further that, although obtained so regularly and abundantly in cultures

Fig. 1. Wrinkled Type of Beet Leaves

Not curly top, but having in the sieve tubes occasional groups of the bacterial bodies found in great abundance in curly top beets.

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[1] Smith, R. E., and Boncquet, P. A. New light on curly top of the sugar beet. Phytopath. **5**: 103–107. 1915.

——— Connection of a bacterial organism with curly leaf of the sugar beet. Phytopath. **5**: 335–342. 1915.

FIG. 2. SUGAR BEET PLANT TYPICALLY AFFECTED WITH CURLY TOP

from diseased plants or portions of plants, it has not been possible to produce curly top by inoculation with cultures of this organism; that the same organism has been isolated from the surface of beet seed, the surface of normal sugar beet leaves and from the soil about the roots of sugar beets. Also that certain bodies which seem to represent the same organism have been found in great abundance in curly top sugar beets, in the interior of the sieve tubes, accompanying a specific lesion in the phloem, and that similar bodies, in varying but much less abundance, were found in the same tissue in supposedly normal beets or those with various morphological irregularities of the foliage (fig. 1). Whatever may be the entire significance of the organism in question, its peculiarly abundant occurrence in connection with the sugar beet and its apparent relation to curly top have seemed to justify its careful study, and it is the purpose of the present article to describe more in detail the characteristics of this species, to which the name *Bacillus morulans* has been given.

THE DISEASE

The disease of sugar beets called curly top is of annual occurrence throughout the sugar beet growing regions of Colorado, Utah, Idaho and California. The severity of the disease, however, varies greatly from year to year. Some years veritable disasters are produced by curly top, thousands of acres of sugar beets being totally destroyed after all the expense of preparing the ground and planting the crop has been undergone.

Symptoms on leaves

The comparative size of the inner and outer leaves is altered. The inner leaves are dwarfed, the petiole especially becoming shorter and flatter than the normal, while the outer leaves, if already full grown before the disease becomes apparent, maintain their natural size and shape and, for some time at least, their color, although they may finally turn yellow and die prematurely. The first symptom of abnormality plainly visible to the eye is a distinct transparency of the finest venations of the youngest leaves. This transparency starts at the base of the leaf blade. Gradually the abnormality works higher on the leaf until finally the whole leaf is affected. The youngest leaves are first to suffer; the older ones (such as are not already full grown) show the symptoms as their expansion and growth progress. Almost simultaneously with the appearance of the transparency of the veins small warty protuberances appear upon the veins on the under surface of the leaves, eventually even upon those which are of the smallest size visible to the eye (fig. 2). The margins of the affected leaves then begin to curl slightly upward so as to expose the lower

surface. As these symptoms gradually increase, working outward from the innermost to the outer leaves, black spots soon appear in the principal leaf venation and especially in the youngest petioles. These black spots often break out through the cortical parenchyma and epidermis, allowing a black syrupy substance to ooze out, which upon drying produces in severe cases a crusty appearance upon the petioles. The interrupted and irregular expansion of the leaf venation soon brings about a very characteristic leaf curl. This curling for the most part is not parallel with the main midrib but extends more or less along the margin of the leaf and converges at the apex. If the plants are badly affected when very young they may be entirely killed; otherwise when the disease is advanced the outer leaves become yellow and die while the youngest may remain for a long time practically unchanged or occasionally may later resume

FIG. 3. SUGAR BEET ROOT TIPS, SHOWING CHARACTERISTIC BENDING AND SWELLING

growth and become apparently free from the above-mentioned symptoms. The latter represents a condition of recovery from the disease. In such cases the young leaves are also dwarfed but the venation is normal, the warty protuberances are absent and the leaf does not curl. In this condition, however, the whole leaf is of a denser green color, harder and thicker. The beet seems to have undergone a struggle similar to that which mesophytic plants undergo when they are transplanted to a desert habitat. In such cases the roots may develop to normal size even when the tops are much suppressed.

Symptoms on roots

It is only when the leaves have been thoroughly affected that the roots begin to show abnormality. This is characterized by the multiplication of rootlets. The older ones begin to die while at their base new ones push forth. This continuous destruction of the rootlets brings about a warty appearance, the bases of the masses of rootlets protruding slightly from the surface of the beet. On careful examination it will be observed that each rootlet, after it reaches an appreciable length and before it dies, has

several abnormal bendings; the angle of each bend is slightly swollen and if the rootlet is far advanced the swollen region appears to be necrotic (fig. 3). When the main root is cut transversely the successive rings of vascular tissue appear discolored. On careful examination it will be observed that the phloem is the only part of the vascular system which suffers severely. This phloem discoloration is observed more or less throughout the whole system in the veins as well as in the roots. This, however, only becomes apparent to the naked eye when the disease has reached its severest aspect.

Cause of the disease

It was E. D. Ball[2] who discovered that the sting of the insect *Eutettix tenella* Baker is a necessary factor in the causation of this disease. His observations were confirmed by Shaw,[3] and very fully tested and confirmed by Smith and Boncquet. The latter, however, together with Hartung,[4] proved a fact which had previously been suspected, that *Eutettix tenella* is not the fundamental factor in the causation of this disease, but rather must be a carrier of a second factor, presumably a parasitic micro-organism. This discovery made very important a thorough search for and study of all micro-organisms which possibly could be found in connection with the disease, and it is with this portion of the study of curly leaf that the present article has to do.

BACTERIOLOGICAL INVESTIGATIONS

The methods and detailed results through which the conclusion was reached by the writer that *Bacillus morulans* inhabits constantly and specifically plants affected with curly top, as well as occurring in certain other situations may first be described. It was decided at the outset to make a very thorough and accurate search for any organism which might be present in the tissues of plants affected with the disease. The unfavorable or at best uncertain results reported by previous investigators along this line led to the belief that the problem would be a difficult one,

[2] Ball, E. D. The beet leaf hopper. Utah Agr. Exp. Sta. Ann. Rept. **16**: 16. 1905.

——— The Genus Eutettix. Proc. Davenport Acad. Sci., **12**: 41 and 84. 1907.

——— The leaf hoppers of the sugar beet and their relation to the "curly leaf" conditions. U. S. Dept. Agr. Bur. Ent. Bul. 66, pt. 4. 1909.

[3] Shaw, H. B. The curly top of beets. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 181. 1910.

[4] Boncquet, P. A. and Hartung, W. J. The comparative effect upon sugar beets of Eutettix tenella Baker from wild plants and from curly leaf beets. Phytopath. **5**: 348–349. 1915.

but at the same time a study of the nature of the disease had led to a very strong feeling that some parasitic micro-organism, of which the insect *Eutettix tenella* was presumably a carrier or secondary host, must be involved in this disturbance. Assuming then that the sought-for organism would be an obscure one and difficult to demonstrate by ordinary cultural or histological methods, various special and somewhat elaborate culture methods were attempted.

Preparation of media

The following media which seemed most promising for this purpose were prepared:

Filtered beet juice. For this purpose the plants from which the juice was desired were washed as thoroughly as possible in sterilized water and then ground fine in a meat grinder. In most cases 100 cc. of distilled water was added to each 100 grams of beet pulp and the mass was then covered and left standing for two hours. The crushed material was subsequently put into clean cheesecloth and the juice squeezed out in a press. The juice thus obtained was subsequently diluted twice its volume with n 6 salt solution. Various degrees of dilution have been employed, however from the original juice up to about ten to one, either in salt solution or water. In some cases the solution was then titrated and brought to the neutral point of phenolphthalein with sodium hydroxid. This juice was now clear, slightly brown and passed easily through a common filter paper. After it had been filtered through paper it was passed through a medium-dense Berkefeld filter candle for purposes of sterilization. An apparatus was especially devised for this purpose, a form of which is described in another article in the present number of Phytopathology. In order to be sure that no contamination had occurred during the manipulation, the tubes after filling were kept in the incubator for two days at 30°C. It is believed that this apparatus and method is worthy of considerable employment in the preparation of culture media for use in plant pathology.

Aseptic, unheated beet slices. These were prepared in the following manner. Sound healthy beets were selected, thoroughly cleaned and immersed in boiling water for three minutes, in this way sterilizing the surface but not heating or changing in any manner the tissues deeper in the beet. They were then cut into slices with a carefully sterilized knife. Each slice was then put into a sterile petri dish into which previously ordinary nutrient agar had been poured.

Beet broth. Three hundred grams of beet leaves were cut into small pieces and boiled for an hour in 0.5 litre of water. Water was then added to make up to 1 litre and left standing for two hours. It was then filtered through cotton and 500 cc. of this beet extract added to 1 litre of Liebig's broth. The Liebig's broth had previously been prepared in the following way: 2 grams of Liebig's extract, 10 grams of Witte's peptone and 5 grams of sodium chlorid were added to 1 litre of water. This medium was subsequently neutralized to phenolphthalein with sodium hydroxid and after addition of the beet juice was brought up to 0.5 per cent acidity with malic acid. The same medium was also prepared with an increased proportion of beet extract.

Artificial media. A protein- and peptone-free medium was composed with the supposition that the organism did not attack the higher nitrogen compounds of the beet. Therefore several of the amino acids were used as the nitrogen supply. Alanin, leucin and tyrosin were used. Asparagin, although not an amino acid was also considered a possible favorable source of nitrogen for the parasitic organism. All these compounds were used in a dilution of 0.5 gram to 1 litre of water. The necessary minerals were added in the following form and proportion:

Magnesium sulfate	0.2 gram
Ammonium phosphate	0.5 gram
Potassium nitrate	0.2 gram
Calcium hydroxid	5 cc. of a saturated solution
Ferric chlorid	trace

These artificial media were sterilized in the Arnold sterilizer for fifteen minutes upon three consecutive days. Special glycerin and glucose media were also prepared. For this purpose 1 per cent glycerin was added to a part of the asparagin medium. So also 5 per cent glucose was added to another portion. The glucose medium was especially used for anaerobic purposes.

Other media. Ordinary media such as nutrient bouillon, potato glucose bouillon, bean pods, milk, litmus whey, nutrient agar and nutrient gelatin were prepared according to the standard methods.

Methods attempted for separating parasites from the plant

In order to separate the assumed parasites from the plant and obtain them in pure culture the following technique was used:

Surface-disinfected plant parts placed in culture medium. The usual method employed in this sort of work consists in soaking the tissue to be employed for a given length of time in mercuric chlorid and then washing off the same with sterilized water before placing the tissue in the culture medium. A need of standardizing this method was felt, inasmuch as there is no assurance, as it is usually described, whether on the one hand the disinfection was sufficiently thorough to kill all surface organisms or whether on the other hand the material was washed sufficiently to remove all the mercury and prevent its being carried over into the culture medium. The method consists in dry sterilizing a number of cotton-plugged flasks of 50 cc. capacity or any other desired size. At the same time larger flasks, likewise cotton-plugged and filled with distilled water, are made sterile in the autoclave. Other requisites are supplies of 95 per cent alcohol and 1 to 1000 solution of mercuric chlorid in water. The material from which cultures are desired, after thorough wiping with cotton swabs in 95 per cent or absolute alcohol in a photographic tray, is cut into convenient sized fragments, but no smaller than necessary. These are placed in one of the empty sterilized flasks and covered for a moment with the alcohol for the purpose of removing air bubbles. The alcohol is immediately poured off again and the flask nearly filled with mercury

solution so that all the material will be submerged. This is allowed to remain for the desired length of time, depending upon the nature of the tissue. The petioles and main veins of sugar beet leaves, especially fairly old leaves, will usually stand twenty minutes, but with leaf blades and other more delicate material ten minutes has been found the maximum time which can be used without too severe burning. Cut surfaces will naturally absorb more of the solution than those protected by the natural covering of the plant, and this can be taken into account both in consideration of the length of time which the tissue will stand without being burned by the mercury and also the time necessary for washing it out again. On this account it is best to cut the tissues as little as possible before disinfecting. After the desired time has elapsed a piece of brass wire gauze, bent to form a cap over the mouth of the flask, is sterilized in the flame, placed in position and the mercury poured off. The flask is then filled with sterilized water from the large flask and the water of this first washing, after having the material well shaken up in it, is poured off immediately and more water poured in. The process of pouring off and refilling is then continued at gradually increased intervals; the length of each must depend upon the nature of the material. It was found, however, that if the amount of material in the flask is comparatively small in proportion to its capacity, which should always be the case, six changes of water, extending over a period of two hours, is amply sufficient in every case. In this case the first five changes can be made during the first hour and the last one at the end of the second hour. If one wishes to practice extreme caution the mouth of the flask may be flamed and the cotton stopper replaced after each change of water, but this has not been found necessary so long as the amount of water is sufficient to thoroughly submerge all the material. In our work the wire screen is usually left over the mouth of the flask and this is freshly flamed before each change of water. After the process is completed the material is taken out of the last water with flamed forceps, broken into small pieces if necessary and thoroughly crushed with the same instruments and dropped into the culture liquid.

Piece cultures. It was thought that a gradual adaptation from the plant in which the organism is living to the medium in which it was attempted to grow it might be necessary to insure success; therefore the diseased tissue was so transferred as to disturb as little as possible the cells of the beet. For this purpose glass tubes were drawn out to 2 mm. diameter. After sterilizing by heat they were aseptically inserted into the diseased regions of the beet to a depth of 1 cm. The tube was then withdrawn, bringing with it a portion of the beet tissue and the terminal part containing the tissue was carefully broken off with sterilized forceps

and dropped into the medium. In this way both ends of the tissue slightly protruded from the glass tube and came into direct contact with the culture medium. The slow diffusion of the latter was supposed to secure a gradual change of habitat in such a way as not to hinder too severely the growth of the parasite. Tissue was thus removed from the petiole, from inside the root and from the larger veins of the leaf, after surface sterilization with a flame or boiling water, afterwards cutting into the interior with a sterilized knife and then introducing the glass tube to take out a small core of tissue.

Results of isolation experiments

The various special methods described were carried out very carefully. The result was that in almost every instance cultures from curly top tissue in all the various media described, and especially those which contained glucose, showed a heavy growth after twelve hours of incubation at 20°C. This result was practically uniform wherever diseased material had been used. Occasionally growth also appeared in cultures from supposedly normal plants, but in by far the great majority of cases such cultures remained sterile. This seemed to indicate that the organism was not peculiarly difficult to isolate, judging from its abundant growth on such a wide variety of media. Nevertheless a painstaking work was undertaken in order to complete the thorough study which had been planned. The anaerobic cultures also proved to be invaded by the same organism. Here, however, the growth was less abundant and extremely slow. Several days elapsed before any colonies were visible. A great deal of effort was further spent on work with all kinds of media but always the same organism grew abundantly. Contaminations naturally occurred now and then but the fact was most decidedly apparent that the one species announced by Smith and Boncquet predominated in the tissues of curly top plants to the practical exclusion of all others. The special culture methods described above are given in some detail, inasmuch as they may contain suggestions of value in similar work. Having found that this organism grew so easily and abundantly upon ordinary media, the use of special preparations was abandoned in the attempts to isolate the organism from plant tissues and the work was carried on entirely with standard bouillon to which 5 per cent glucose had been added. The object of the glucose was to promote the growth of the characteristic zoogloeae of this organism, rendering its identification in the original tubes easy without plating. By occasional plating, as a check on the work, it was soon possible to identify this organism very accurately by microscopic examination of tubes which showed the characteristic ring formation at the surface of the liquid. The

TABLE 1

Results of bacterial isolation experiments by cultural methods

(Tubes incubated at 28°C. *Disease* means curly top)

MATERIAL	NUMBER OF TUBES	RESULT
Petioles of curly top beets	6	5 tubes developed *B. morulans* within two days
Petioles of normal beets	6	Tubes remained clear for a week, when they were discarded
Petioles of diseased beets	9	8 tubes developed *morulans*
A leaf showing curly top symptoms on half of blade and in corresponding half of petiole. Other side appeared normal. This material from affected half of petiole with black streaks	5	All developed *morulans*
Corresponding half of blade	3	2 tubes developed *morulans*
Normal-appearing half of petiole	6	5 tubes developed *morulans* two days later than those from blackened part
Normal-appearing side of blade	3	No development
Petiole of a diseased center leaf	4	3 with *morulans*, 1 doubtful
Petioles of good-sized leaves from 5 different normal-appearing beets	10	2 tubes from 1 leaf both with *morulans;* others all clear
Typically diseased leaf with very slight dark streaks in the petiole	6	All developed *morulans*
Badly affected petiole of same beet. Pieces cut out with flamed scalpel	4	All developed *morulans*
Petioles of 4 beets from insect-proof cage. No sign of disease	8	Tubes from 2 plants remained clear; those from other 2 became slightly cloudy after several days, but no *morulans*
Petioles of 2 slightly diseased leaves. Pieces cut out with flamed scalpel	13	All tubes apparently containing pure cultures of *morulans*
A yellowish aster leaf	2	Remained clear
An old yellowish beet leaf without curly top. Tissue still sound	4	Some fungous growth. No *morulans*
Leaves of a somewhat abnormal-appearing beet but not with curly top	6	Some growth, but no *morulans*
Healthy-appearing leaves of aster, chrysanthemum, dahlia, tomato, bean, lettuce and radish	24	11 tubes with fungi and bacteria, remainder clear. No *morulans* found
Petiole of typically diseased leaf, no disinfection	6	Very abundant growth of *morulans* intermixed with other organisms

TABLE 1—*Continued*

MATERIAL	NUMBER OF TUBES	RESULT
Petiole of slightly diseased leaf	2	Both very abundant *morulans*
Blade of same between veins	2	Both remained clear
Typically diseased leaf; scraped out interior portions of petiole with flamed scalpel after cleaning off epidermis	4	3 tubes developed *morulans*. 1 doubtful
A young leaf visibly affected on one side and very slightly at the base of the other side. Tissue taken from the most diseased side at base	4	All developed *morulans*
Terminal portion of diseased side, less visibly affected	4	2 developed *morulans;* 2 clear
Slightly affected base of other side of same leaf	4	1 developed *morulans;* 3 clear
Not visibly affected terminal portion of last	4	All remained clear

NOTE.—The last four are from the leaf illustrated in Phytopathology **5**: 106. The most elaborate precautions were taken to secure perfect surface disinfection and avoid contamination. These tubes in which growth appeared were plated out and found to contain pure cultures of *morulans*. The leaf was perfectly sound, showing only a slight roughening of the veins on the affected portion.

appearance to the eye of this ring, supplemented by microscopic examination, finding it to be composed of the characteristic zoogloeae, supplemented by occasional plating, is amply sufficient to identify this organism. A number of typical examples of isolation experiments with sugar beets are shown in table 1.

Several hundred illustrations similar to those shown in table 1 might be given. The results varied somewhat with the perfection of technique

TABLE 2

Bacillus morulans upon sugar beet seed

MATERIAL	NUMBER OF TUBES	RESULT
Beet seed imported from Germany, 1 dropped into each bouillon tube, with no previous treatment	10	At least 7 developed an abundance of *morulans*, mixed with other organisms
Similar seed previously soaked for twenty minutes in mercuric chlorid and washed in sterilized water	10	All clear

and in individual cases, but a mass of evidence was collected to indicate that this organism exists regularly in the interior of the foliage of sugar beets where the visible symptoms of curly top occur and that it does not develop in cultures from normal foliage or even the normal-appearing portions of partially affected leaves; also that it does not occur in the interior of beet leaves which may be yellow or sickly from ordinary causes.

Cultures from seed. Many attempts similar to those shown in table 2 were made to isolate the organism from sugar beet seed. The uniform result was that almost every unsterilized beet seed dropped into a tube of bouillon developed a very luxuriant growth of ***Bacillus morulans.***

TABLE 3

Bacillus morulans from soil

MATERIAL	NUMBER OF TUBES	RESULT
Pinches of soil from about the roots of a diseased beet	4	*Morulans* was abundant in several of the tubes
Pinches of soil from about the roots of a normal beet in insect-proof cage	4	Some *morulans* present in the mixed growth resulting

Cultures from soil. That the organism is present in some soils is indicated by the data presented in table 3. The work was rather crude but certainly *B. morulans* was abundant in the soils examined.

Cultures from unsterilized foliage. Cultures made from unsterilized leaves of the sugar beet (table 4) show that the organism is common as a saprophyte upon the leaves of the plant, but in all cases when leaves similar to these were thoroughly disinfected no growth was obtained.

TABLE 4

Cultures from unsterilized foliage

MATERIAL	NUMBER OF TUBES	RESULT
Leaves of normal beets	10	Several contained an abundance of *morulans*

Unsterilized leaves of many other plants were also tried but the resulting growth was so mixed that no safe conclusions could be drawn. The only certain developments of *morulans* occurred in tubes inoculated with pieces of chrysanthemum leaves.

Cultures from sugar beet leaves with types of disease other than curly top. The fact that bodies resembling bacteria have been seen with the microscope in sugar beet leaves not affected with curly top, but affected with

various types of morphological abnormality, has been referred to in our recent article in Phytopathology. These various foliage types have never been definitely classified or described nor has the extent of the occurrence of this organism in other tissues been learned. In this cultural study,

FIG. 4. MOTTLED LEAF OF SUGAR BEET

however, two at least of these forms gave the organism in abundance (table 5). In one of these, which we shall call mottled leaf (fig. 4), the blades of the leaves are strongly mottled with green and pale areas in a very characteristic manner. In the other type which we call black edge or black tip (fig. 5), the green leaves begin to die and turn decidedly black

about the margin or at the tips, with a yellow band of tissue between the black and the normal green. In some such leaves the development is very much suppressed, even to a point where the leaf consists of scarcely more than a petiole with a small blackened tip.

FIG. 5. BLACK EDGE AND BLACK TIP OF SUGAR BEET LEAVES
Black tip (left) and black edge of sugar beet leaves

Relation of mottled leaf and black edge to curly top. For some time it was believed that these conditions represented types or stages of curly top. Later, however, it was found that they occurred under circumstances which made this seem practically impossible. Further, when the experiment was tried of placing *Eutettix tenella* upon such plants the typical disease developed promptly.

TABLE 5

Cultures from sugar beet leaves with types of disease other than curly top

MATERIAL	NUMBER OF TUBES	RESULT
Yellowish area of young "black edge" leaf taken from between black and green portions. Thoroughly disinfected	2	Both gave a strong growth of *morulans*
Similar to last, but not disinfected	4	Very vigorous and apparently nearly pure growth of *morulans*
Similar material, disinfected	4	All with *morulans*
Similar material, disinfected	4	All with *morulans*
Typical "mottled leaf;" not disinfected. Blade, petiole and veins	9	All produced *morulans* in abundance
Petiole of "black edge" leaf, disinfected	4	All appear to have pure cultures of *morulans*
Petioles of normal appearing leaves from healthy plant. Very carefully disinfected	5	All tubes clear
Petiole of decidedly "mottled leaf." Inner tissue removed with flamed scalpel	4	Very vigorous growth of *morulans*

Further study of the organism, which was uniformly present in diseased beets

Although the organism was able to grow most abundantly on the common culture media, the peculiarity of this growth under all circumstances was of such a nature that for some time a continual contamination was suspected. Each separate colony seemed always to have two kinds of bacteria, very distinct in form. Very active bacteria were always observed at the edge of the colonies, while capsultated bacilli were generally observed in the middle. Therefore, before any further study of the organism was taken up, repeated efforts were made to separate these two widely distinct forms. For this purpose, the calcium carbonate and the India ink method for separating the individual organisms previous to plating them were resorted to.

Calcium carbonate. To 10 grams of calcium carbonate enough water was added to form a milky paste. This was subsequently introduced into a 200 cc. Erlenmeyer flask and sterilized in the autoclave. After the necessary cooling several young colonies of the bacteria were introduced into the semi-liquid mass and shaken for two hours so as to separate each individual organism from the other. From this paste, several plates were poured in the usual manner. They were incubated at 37°C. and closely examined as soon as any sign of development occurred.

India ink method. For this purpose special Chinese ink, prepared by Grübler, (*Punktlusche*) was used. A 15 per cent nutrient gelatin was made and poured into clay-covered petri dishes. Special care was given to prevent condensation water from flooding the medium. The Grübler's ink was diluted twenty times with n/6 glucose solution and sterilized in the autoclave. In a sterilized, empty petri dish ten drops of the ink were put in a row. The first drop was inoculated with a small amount of bacteria from a twelve-hours-old streak culture. The bacteria were thoroughly mixed with the ink of the first drop. Then a loop of this was transferred to the second drop and also thoroughly mixed. This transfer was repeated in the same way with the remaining drops in the dish. From the tenth drop, with a sterilized drawing pen, a small amount was taken. Small dots were made with the pen on a gelatin plate in such a way that the surface pellicule of gelatin remained uninjured. These ink dots were left to dry for two minutes then covered with a sterilized cover-glass. A small drop of immersion oil was subsequently applied to the cover-glass and the whole petri dish was brought to the microscope for examination. Each black point was then examined with microscope until one was found which contained one single organism. The organism appeared as a translucent dot on a black field. Its development was closely followed; the first division was distinctly noticed after half an hour; it multiplied rapidly; all the individuals were motile; they liquefied the gelatin slightly and moved about very briskly in the liquid under the cover-glass. After six hours some of the organisms became sluggish and gradually lost their motion. They increased in size and formed a capsule. Repeatedly they divided in the same capsule, stretching the jelly-like membrane more and more. The newly formed organisms within the original capsule also encapsulated in their turn (figs. 6 and 7). At the same time the individuals on the rim of the colony multiplied and remained motile. The double form of the bacillus was in this way clearly explained and proved.

IDENTITY OF THE ORGANISM FOUND IN CURLY TOP BEETS

A study of the literature of the subject shows that the greatest similarity to our organism of any described species is presented by that described by Arthur and Bolley[5] as *Bacterium Dianthi* as the cause of a leaf spot of the carnation. In its morphology, so far as described by these writers, this organism is very similar to ours, the resemblance being made pronounced by the development of characteristic zoogloeae. In biological behavior, however, the two organisms cannot be accurately compared, since the

[5] Arthur, J. C. and Bolley, H. L. Bacteriosis of carnations. Indiana Agr. Exp. Sta. Bul. 59. 1896.

work of Arthur and Bolley was carried on at a time when bacteriological technique was not standardized upon modern lines. One noticeable difference exists in respect to growth upon an acid medium, *B. Dianthi* being said to grow best under such circumstances, which is not the case with our organism. The description of the bacterial organism given by Arthur and Golden[6] and again by Miss Cunningham[7] as the cause of the so-called Indiana sugar beet disease, is similarly subject to uncertainty, but if this work was accurately done the organism must certainly have been different from ours in that it is said to be a particularly active gas former, which feature is totally lacking in our organism. It seems proper to mention here, however, the fact that Professor Arthur in a recent personal letter states that the accuracy of all this early work performed under his direction is open to some doubt on account of the undeveloped condition of bacteriological technique at the time and he expresses the opinion that the organisms found by Bolley, Miss Golden and Miss Cunningham were very likely identical.

The organism described in the unpublished work of Schneider[8] as *Bacillus californiensis*, which was isolated from curly top beets in California, seems again in its morphological characteristics to be entirely similar to ours and we feel little doubt that Schneider and the present writer had the same organism before them. Schneider found his organism also very abundant in sugar beet soils and upon the surface of the plants. He attributes a stimulative effect to this species, when applied in pure culture to sterilized beet seed or to the foliage of young plants.

The organism described by Düggeli[9] as being abundantly and often exclusively present upon the surface of various plants and seeds, seems also very similar to ours in form and size, formation of zoogloeae, color, saprophytic habitat and most biological characters. This was named by Düggeli *Bacterium herbicola aureum*, but "said to be the same as the *Bacillus mesentericus aureus*, isolated by Winkler from the surface of plum leaves." The latter statement confuses the identification.

DESCRIPTION OF THE ORGANISM

Summing up the whole situation, we feel justified in describing our organism as a new species on account of the incomplete and doubtfully

[6] Arthur, J. C. and Golden, K. E. Disease of the sugar beet root. Indiana Agr. Exp. Sta. Bul. 39, pt. 3: 54. 1892.

[7] Cunningham, C. A. A bacterial disease of the sugar beet. Bot. Gaz. **28**: 177–192. 1899.

[8] Schneider, A. The California beet blight. Spreckels Sugar Co. Exp. Sta. Rept. **23**: —. 1906. (Unpublished.)

[9] Centbl. Bakt. II, **12**: 602 and 695; **13**: 56 and 198. 1904.

accurate descriptions of those species which more or less resemble it, and the fact that none of them corresponds throughout.

Bacillus morulans n. sp.

Morphology

Vegetative cells. Grown in Liebig bouillon for sixteen hours at about 20°C., oval to short rods, single or in pairs. Grown at 37°, short rods in pairs or in short chains.

Size. Length 1.5 μ; breadth 0.9 μ; extreme length from 1.5 to 2 μ.

Capsules. Easily observed in 1 1000 glycerin bouillon after twenty-four hours and also in milk media (figs. 6 and 7).

Motility. Very active on agar and in bouillon, when grown at 37° for twelve hours.

Flagella. Stained by Zettnow's method; four long peritrichial flagella (figs. 6 and 7).

Pleomorphism. Cocciforms observed in glucose bouillon tubes and blood serum media after thirty days.

Stain. Easily with watery fuchsin, decolorized by Gram's method.

Cultural features

Gelatin plate neutral to phenolphthalein. Form, round to irregular; surface elevation, flat to convex contoured; internal structure, refraction strong, hyaline, moruloid; zoogloeae very marked; edges, entire to undulate; optical characteristics transparent to butyrous; consistency, viscous. Each colony is surrounded with many secondary colonies, appearing as small, oily drops of high refractive power. The appearance of zoogloeae is very noticeable in the middle of the colonies.

Gelatin plates 1.5 acid to phenolphthalein. The entire mass is a zoogloea, lobed and irregular in outline; the colony is slightly colored; orange-yellow, no surrounding colonies noticed.

Gelatin streak. After five days: growth, linear; margin, continuous; surface relief, flat to convex; light transmission, butyrous; color, yellow-orange; luster, glistening; consistency, viscous. The water of condensation has a yellow sediment.

Gelatin stab. After twenty-two hours, top growth: size, 5 mm., irregular contoured, pulvinate to capitate, light orange in color; viscous in consistency, luster shining.

Gelatin stroke. Filiform to slightly beaded. After fifteen days, liquefaction of medium, crateriform with a yellow sediment. After twenty days, liquefaction stratiform; yellow pigment, decreased by absence of oxygen.

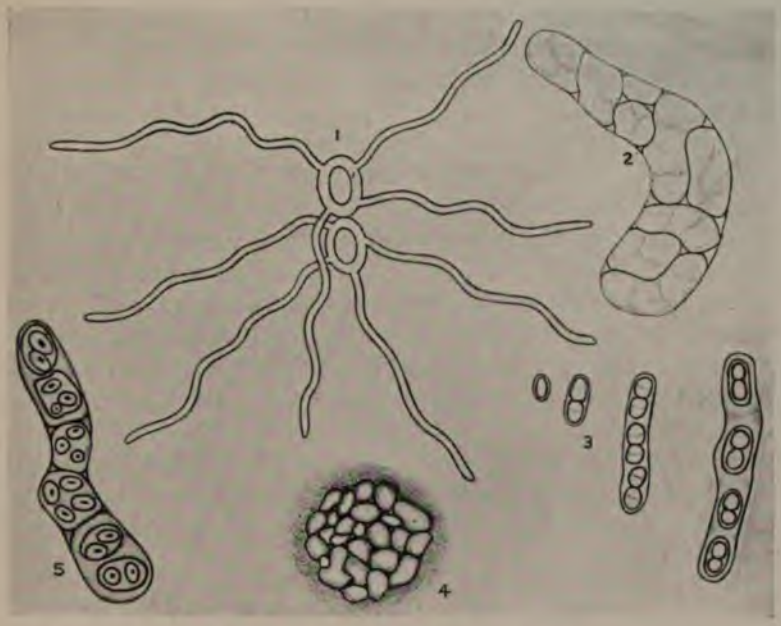

FIG. 6. MORPHOLOGY OF BACILLUS MORULANS

1, A chain of two individuals, showing flagella.

2, *3*, *5*, development of capsule and zoogloea from one individual. *4*, mass of zoogloeae.

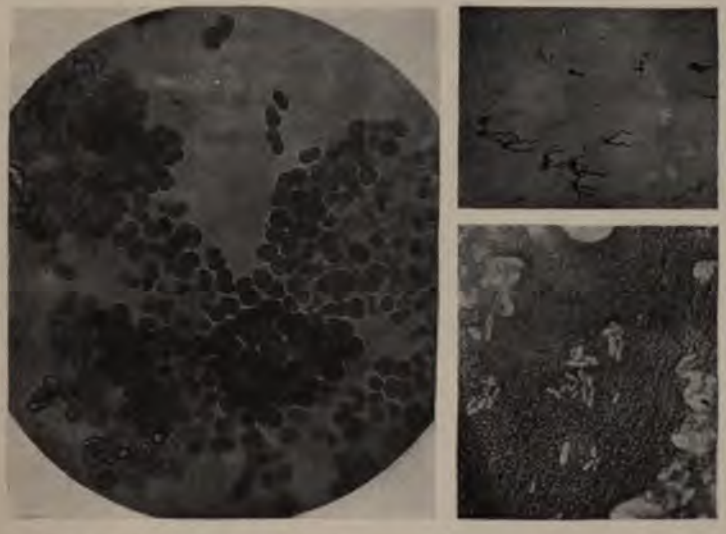

FIG. 7. BACILLUS MORULANS, SHOWING CAPSULES AND FLAGELLA

Agar. The colonies are extremely variable according to the density of growth, the moisture and the temperature.

Milk. Peptonisation of casein in fifteen days at 37°. The reaction is alkaline to Azolitmin.

Litmus whey. Remains clear, alkaline reaction.

Bouillon tubes. Opacity begins after eight hours at 37°, a pellicule forms in twenty-four hours or less. The color of the pellicule and the ring is dull, soft gray; thick, viscous and consists of conglomerate zoogloeae. These are generally oval, but may be linear and all united in chains.

Deposit, forms after two days incubation at 37°. Deposit is in the beginning slight, and finally yellow. The amount of deposit and the intensity of the color increases, however, rapidly. After ten days, a decidedly deep yellow-orange has developed. The deposit is compact and viscid on agitation.

Potato streak. After twenty four hours at 37°. Size, 2 mm.; sharp, linear; margin, continuous; color, yellow, homochromous; luster, glistening; texture, homogeneous. No liquefaction of potato and no gas formation.

Physical and biochemical features

Reaction. In carbohydrate-free media the reaction is alkaline; in carbohydrate media, the reaction is acid, except in lactose, where the reaction is slightly alkaline. See table 6.

Nitrate Liebig broth. After twenty-four hours at 37°, strongly reduced to nitrite.

Indol. Not produced in peptone solution after ten days.

Optimum temperature. 37°, measured by the amount of acid produced in 1 per cent glucose after five days. Acidity was 2.5.

Thermal deathpoint. Six-hours culture in bouillon; 54°C. in ten minutes.

Carbohydrate fermentation. Shown in table 6.

Resistance to mercuric chlorid. Six-hours culture on bouillon agar streak killed in 1 25,000 to 1 30,000 in ten minutes.

Relative growth in acid and alkalin media. Determined by the appearance of cloudiness in the tubes. Grows best on neutral or slightly alkalin media. Five per cent in acid apparently stops all growth; 7 per cent in alkaline: same.

Gas production. No gas is produced. See table 6.

Relation to free oxygen. Aerobic; facultative anaerobic.

TABLE 6

Carbohydrate fermentation of Bacillus morulans

(Incubation: 37°C., medium neutral to azolitmin)

MEDIUM: 1 PER CENT PEPTONE AND 1 PER CENT CARBOHYDRATE	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 5 DAYS	ACIDITY AFTER 5 DAYS	GAS AFTER 5 DAYS	DEPOSIT	FILM	RING	REACTION	
											Closed arm	Open arm
						$\times 10^{-5}$						
Dextrin	0	0	AB*	AB*	AB*		0	p	a	a	acid	alk.
Inulin	0	0	0	0	0	0	0	a	a	x	0	0
Amygdalin	0	0	0	0	0	0	0	a	a	a	0	0
Salicin	A	A	A	A	A	1.8	0	p	a	a	acid	acid
Glycerin	0	0	0	A	A	0.1	0	a	a	b	acid	acid
Lactose	0	0	B	B	B	0.2	0	a	a	a	basic	basic
Laevulose	A	A	A	A	A	2.3	0	b	b	p	acid	acid
Galactose	A	A	A	A	A	2.1	0	b	b	p	acid	acid
Dextrose	A	A	A	A	A	2.5	0	b	b	b	acid	acid
Saccharose	A	A	A	A	A	1.6	0	p	b	p	acid	acid
Mannit	A	A	A	A	A	1.3	0	p	a	x	acid	acid
Maltose	A	A	A	A	A	1.3	0	p	a	p	acid	acid
Rhamnose	A	A	A	A	A	1.3	0	p	a	x	acid	acid

NOTE.—*A*, acid; *B*, basic; *p*, permanent; *b*, abundantly present; *a*, absent; *x*, more or less present; *, acid on top and basic in tube.

Pathogenesis

One loop from a twelve-hours-old streak culture on bouillon agar introduced intravenously in a rabbit, caused death within twenty-four hours.

On *Dianthus incarnata*. The young unfolded leaves, when unrolled and covered with an abundant suspension of bacteria, developed small necrotic regions. The necrotic regions are watery and translucent on the edges, slightly elongate or irregular in outline, following the venation. The inside of the necrotic regions is slightly brown.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

A NEW APPARATUS FOR ASEPTIC ULTRAFILTRATION

RALPH E. SMITH

WITH TWO FIGURES IN THE TEXT

In various investigations of so-called nonparasitic or physiological plant diseases, as well as in numerous animal diseases in which the presence of an ultramicroscopic organism is suspected, the juices or body fluids of affected plants or animals have often been subjected to filtration through Berkefeld, Chamberland and similar filters in order to remove bacteria and other organisms of microscopically visible size. While inoculations with such filtered juice have been made frequently, and in some cases (tobacco mosaic) with positive results, the possibility of demonstrating the presence of a parasite in the filtered juice by its possible growth when all other organisms are excluded, seems to have received little attention so far as plant diseases are concerned.

Another object in producing an aseptically filtered juice is that of obtaining a sterile, unheated plant extract as a culture medium, as mentioned by Mr. Bonequet in another article in this number of Phytopathology. In either case the juice may be varied in concentration or reaction, any desired substance which will pass through the filter may be added to it, and in many ways use may be found in the investigation of plant disease for aseptically filtered and preserved or cold-sterilized juices. Various devices for this purpose have been described but all of them, so far as the writer is aware, are clumsy and of very doubtful efficiency. Those in which the candle and the liquid to be filtered are in a tube directly above the receptacle or outlet for the filtered juice are especially undesirable, since the slighest leakage may result in contaminating the filtrate with the unfiltered juice and thus defeating the whole object of filtration. Rubber stoppers are always open to suspicion. Arrangements in which the filtrate is caught in an open or a cotton-stopped vessel and then poured into the culture tubes in the open air are certainly far from safe. Those in which the liquid is forced through the candle from the inside outward are objectionable on account of the quick coating over of the inner surface with sediment, which, on the outside, may be mostly brushed off. In the apparatus illustrated here the only possibility of contamination is from the air and cannot occur from the unfiltered juice. In other words, it is absolutely certain that nothing

which is in the original juice can get into the filtrate except by passing through the pores of the candle.

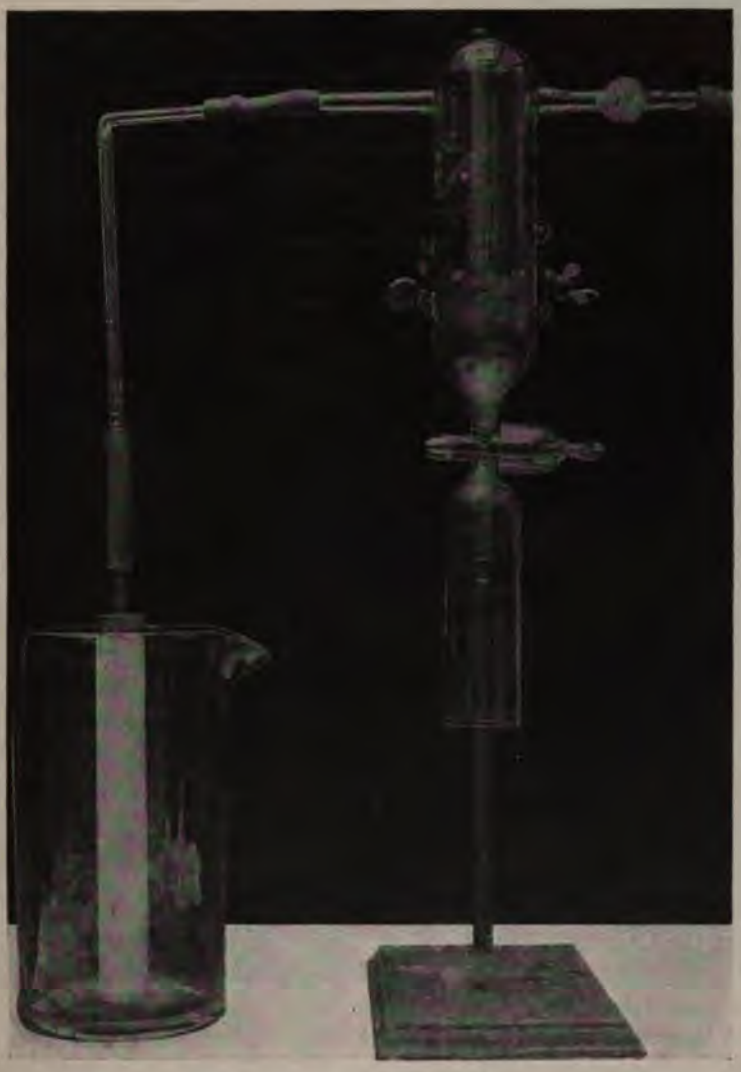

FIG. 1. FILTER APPARATUS, BEFORE WRAPPING

In this apparatus the liquid is drawn up through the filter into the large upper tube and run off below by means of the stop-cock and outlet into sterilized test-tubes. Before commencing operations the two joints

in the line of tubing from the candle to the upper reservoir are wrapped closely with cotton, extending beyond the ends of the rubber connections and for about an inch down over the candle itself. Over these are put again still longer cotton wrappings and finally a layer is put on covering the whole line completely from a point two inches below the upper end of the candle up to the reservoir. The whole region around the stop-cock is similarly wrapped with several layers of cotton, with a large plug of the same material in the lower opening and a wrapping over this and up around the tube. It is the intention that all the joints shall be air-tight and in this apparatus the number of joints and chances of contamination are less than in most similar devices. (Compare for example the arrangement figured on page 65 of Marshall's Microbiology.) The cotton wrappings are added, however, as an extra precaution.

After the apparatus has been put together and wrapped it is sterilized in the autoclave. At the same time a supply of cotton-plugged test-tubes is sterilized by dry heat. The apparatus is then set up on a table convenient to a gas burner and some of the juice to be filtered poured into the beaker, taking care not to allow the cotton at the upper end of the candle to touch the liquid. The water vacuum pump is started, using as little suction as possible, and allowed to operate until a supply of the filtered juice has been collected in the reservoir. If the surface of the candle becomes too much clogged it may be cleaned with a soft brush, but it is desirable before commencing the filtration to remove as much solid material from the juice as possible by filtering through cloth, paper and sand. As soon as enough juice has been filtered the cotton plug closing the bottom of the main tube is removed; one of the sterilized test-tubes is held in one hand, the plug removed and discarded, the tube thoroughly flamed several inches down from the top and then passed up into the lower end of the apparatus below the outlet tube. With the other hand the stop-cock is turned and some of the liquid run into the tube. After shutting off the cock, another tube is taken from the basket with that hand, while the tube just filled is withdrawn and its open end held in the flame. The plug of no. 2 is now transferred to no. 1, both tubes constantly flamed, no. 1 is laid aside and no. 2 filled, continuing the process with as many tubes as desired.

The apparatus figured is 14 inches long over all, and holds 200 cc. of liquid. Different sizes may of course be made and it is very probable that some of those interested in the matter may be able to suggest improvements upon the apparatus. A continuous glass tube might be used between the reservoir and filter candle, thus eliminating one joint, but it has been thought that the increased liability of breakage would more than offset any disadvantage. It might also be possible to cement

the joints in this tubing with some air-tight material, and thus eliminate any possibility of leakage at those points. This would leave the stop-cock as the only possible place where anything could get in without passing through the candle. Its efficiency depends upon the tightness with which it fits together and upon its being well greased. Any leakage at this point may be easily detected by the bubbles of air passing up through the liquid.

FIG. 2. METHOD OF USING FILTER APPARATUS

The apparatus shown in the illustration was constructed by the Braun-Knecht-Heimann Company, of San Francisco, after sketches by the writer.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

FACTORS AFFECTING THE PARASITISM OF USTILAGO ZEÆ

F. J. Pieмeisel

Because certain facts seemed to indicate a different life history from that usually credited to *Ustilago Zeæ* (Beckm.) Unger., on corn, field inoculation experiments were carried on during the summers of 1913 and 1914. The results of the experiments were such as to make a more detailed investigation of the spores and sporidia highly desirable.

The works of Von Waldcheim (10), Brefeld (2, 3), Hitchcock and Norton (6), and Clinton (4) have given us the salient points in the life history of this parasite, and numerous other investigators have made additional contributions from time to time, showing that the control of the fungus is a difficult problem. The spores of the smut are widely distributed by the wind and are produced in large numbers throughout the growing season. They are capable of germinating immediately and, in a suitable medium, they produce immense numbers of sporidia which may bud in a yeast-like manner and produce a host of others. The production of the sporidia in large numbers in the field is possible in such places as manure or compost heaps.

Brefeld (2) conducted a few experiments from which he concluded that sporidia are short lived, dying in five weeks when dry. The sporidia therefore have been characterized as being "short lived" and very little is really known concerning the factors affecting their vitality. Nor do we know the fate of spores on the corn used for ensilage. The present investigation was made in an effort to secure more definite information on these points.

INOCULATION EXPERIMENTS

Methods

Pure cultures of the fungus were obtained by the poured plate method, usually on beerwort agar. The colonies were later transferred to agar in tubes. Sporidia from pure culture were then used in the inoculation experiments. Inoculations were made either by smearing the sporidia directly on to the plant parts or by placing them in water and applying this suspension of sporidia by means of a dropper or hypodermic syringe. The hypodermic syringe was used when it was desired to inoculate the very young parts which had not yet been unfolded.

Spores were also used in inoculating the plants—usually they were dusted directly into the tops of the plants or mixed with moist soil and then applied. In a few cases the spores were applied in suspension in water.

Factors affecting infection

During the summers of 1913 and 1914 a total of 2064 plants of Minnesota no. 13 corn were inoculated. In all eighty different series of plants were used in the tests. The highest percentage of infection in any series in the summer of 1913 was 70.8 and in the summer of 1914, 84.2. These results were obtained by injecting suspensions of sporidia in water into the growing point or as near it as possible.

Experiments were made to determine the effect of the following factors on the success of infection: age of the plants; injury to plants; age of the spores and sporidia. Observations were also made on the relation of early planting close planting and soil conditions to the amount of smut present in the fields.

Age of the plants. It was found that successful infection depended very largely on the age of the plants or plant parts inoculated. Healthy, vigorous plants about 2 to 3 feet high are most susceptible. It was very difficult to infect very young or very old plants. These results confirm those obtained by Brefeld (3) and Hitchcock and Norton (6).

Injury of the plants. Experiments performed by Clinton (4) showed that mutilation of the corn plants when about ready to tassel tends to increase their susceptibility. The experiments of the writer likewise showed that injury tends to increase the chances for infection. When young leaves were injured and then inoculated, the resulting infection usually spread from the point of injury as a center. Many of the infected leaf areas, however, never developed sufficiently to produce mature spores. This was probably due for the most part to the rapid maturing of the leaf tissues and the consequent inability of the fungus to spread through these older tissues. Injury is not necessary, however, for successful infection.

Age of the spores and sporidia. The length of time that the spores can withstand unfavorable conditions is important from the standpoint of the propagation of any fungus. Brefeld (2) demonstrated that corn smut spores, eight years old, germinated, but he did not attempt to secure infection with them. The writer obtained spores, five years old, and made field inoculations with them by dusting into the tops of the plants. Six per cent of the inoculated plants became smutted near the point of inoculation. Two per cent of smut also appeared in the check plot but the infected areas appeared on various parts of the plants.

Many fungi when kept in continuous culture on nutrient media for

some time lose their power to infect. Brefeld (2) found that smut sporidia, when kept for a year in continuous culture in liquid media, lost their viability. The writer kept the sporidia in continuous culture on beer-wort agar for eight months. The culture tubes containing the sporidia were then buried in snow out of doors where they remained for a month. Inoculations were then made in the greenhouse on two corn plants. One, a small plant about 2 feet high, became infected in each of the five leaves inoculated. The other, about 5 feet high, was inoculated on an ear and produced a large smut boil. Thus it will be seen that continuous culture of the sporidia on a solid nutrient medium for a period of eight months, followed by freezing for a month, did not destroy their ability to infect. Recently, portions of a pure culture, that had been maintained on beer-wort agar since June, 1913, a period of three and one-half years, were placed in hanging drops of distilled water and also of modified Cohn's solution. In these cultures individual sporidia were probably no longer present but there was a mass of short hyphal threads, apparently resting segments of the germ-tubes of the sporidia. The ends of these hyphal threads were densely protoplasmic and when they were placed in water, or, better still, in a liquid nutrient medium they again produced sporidia by budding. These sporidia continued to bud until the supply of nutrient material was exhausted when they again sent out long thin germ-tubes. The writer inoculated four plants with some of the pure-culture material but has not as yet succeeded in securing successful infection.

Effect of early planting, close planting and soil conditions. Arthur and Stuart (1) report that early planting, close planting and moist rich soil increase the amount of smut. Rainy periods were also closely followed by outbreaks of smut in the corn fields. The following observations made by the writer point to similar conclusions.

The corn used for the inoculation experiments was planted at successive intervals throughout the season so as to have at all times an abundance of plants in the susceptible stage. The plots were carefully examined for smut and it was found that a greater amount of smut was present in the plots planted earlier in the season. The plants in such fields are in the most susceptible condition at a time when the weather is still fairly cool and moist and the conditions for infection very favorable. The later summer weather is likely to be too hot and too dry for successful infection. Late summer with its more frequent rains and cooler weather again brings on a fresh outbreak of smut, the parts most affected being the rudimentary ears, since the rest of the plant is already too mature.

When corn is grown in closely planted rows for green fodder or silage purposes the smut is also more prevalent, probably because the corn in such fields remains succulent for a longer period of time. In one such

field which had been continuously cropped to corn a number of years, there was 25 per cent of smut as compared with 7 per cent in neighboring fields where the corn was 3 to 4 feet distant in the rows and on which rotation had been practiced. Corn on poorly drained soils or on those which are too dry has less smut than that on a rich, moist soil. The former types of soils produce weak plants that mature rather rapidly, while a moist, rich soil produces a very vigorous succulent plant which apparently is also more susceptible to smut. These observations further confirm the conclusions of other writers.

Character of infection

Smut boils are often so generally distributed on a plant as to lead one to suppose that the infection may have occurred on young plants and that the fungus then spread throughout the growing tissues. It is not uncommon to find a single plant on which the tassel, leaves and the primary and rudimentary ears are smutted while adjacent plants are entirely free of smut. It is therefore difficult to explain why the successive infections should all have occurred on one plant. If the smut were systemic such a phenomenon would be easily explained. Brefeld (3), however, concluded from his inoculations that the infection is local.

Field observations. In order to obtain more complete information on this point, the writer made careful observations on plants artificially inoculated in the field. The results showed that, when successful, signs of infection always appear in from ten to fourteen days after inoculation and that the fungus spreads but little in the tissues. On several plants where the inoculum trickled down the sides of the culm, smut boils were produced at a number of points along the line, involving the leaves, the primary ears and rudimentary ears. In nature, similar conditions might easily arise when a spore falls into the water contained in the funnel formed by the unfolding leaves and produces sporidia. The sporidia could then be easily washed out to the various parts of the plant by rain. This is especially probable since the spores of the smut can germinate as soon as mature and parts beneath the point of primary infection are particularly liable to attack.

Brefeld (3) and Kühn (7) both obtained successful infection on a few seedling plants but in all cases such plants were destroyed by the smut. In order to further ascertain the results of early infection, ten very young smutted plants in a fodder-corn field were selected and marked. All of the plants were about a foot high and showed varying degrees of infection. Of the ten plants under observation eight were killed by the smut in less than a month and the other two were greatly stunted. These two plants,

however, produced healthy ears. Furthermore, many plants that were found smutted when quite young matured healthy ears.

Greenhouse inoculations. It has already been stated that in the field the infection of very young corn plants was very difficult. Further inoculations were made in the greenhouse upon germinating seeds. Forty-three seedlings were dipped into a water suspension of sporidia and then planted in pots. None of the plants developed smut although they were allowed to grow for one and one-half months. These results indicate strongly that infection is purely local and not systemic.

THE VITALITY OF SPORES AND SPORIDIA

It is a well-known fact that corn smut spores retain their viability for a number of years, being extremely resistant to unfavorable conditions. Very little, however, is known concerning the vitality of the sporidia and their resistance to unfavorable conditions has hitherto been supposed to be very slight. The writer also investigated the vitality of the spores and sporidia. The effects of the following factors on the vitality of spores were considered: (1) the silo, (2) temperature, (3) carbon dioxide, (4) acids, and (5) seasonal factors. The influence of the following factors on the vitality of sporidia was considered: (1) temperature, (2) desiccation, (3) acids.

Vitality of spores

Since a considerable amount of corn smut must be carried over into silos with the corn in the process of silo filling, it is important to know the fate of such spores. To determine this, spores were collected in quantity and placed in several silos for various lengths of time. A brief summary of the results is given below.

Samples I. These spores, which had just matured, were collected September 11, 1914, enclosed in a cheesecloth bag and placed about one-third of the way down in a wooden silo. A sample of the same lot was kept as a check in the laboratory at room temperature. The spores were recovered from the silo on November 2, 1914, after having been there about seven weeks. The spores were frozen and were, therefore, thawed out gradually in the laboratory.

Sample II. These spores were collected and placed in a brick silo September 2, 1914. The spores were enclosed in a bag and placed about 15 feet from the bottom of the silo and about 2 feet from the side. They were recovered March 4, 1915, after having been in the silo for over twenty-six weeks. The spores when recovered were frozen and therefore the sample was divided into three portions as follows: (1) A portion

was kept frozen; (2) a portion was kept moist at laboratory temperature; (3) a portion was air dried and kept at laboratory temperature. The object was to eliminate, if possible, the method of handling the spores as a factor which might influence their germination.

Samples III and IV. The spores were collected, placed in bags and buried in a silo October 6, 1915. Sample IV was placed about 2 feet from the side and about 8 feet from the bottom of the silo. It was recovered January 20, 1916, and was kept frozen until February 8, 1916, and then kept dry at room temperature. Sample III, which had been placed in the center of the silo, about two-thirds of the way up, was recovered January 15, 1916. It was also kept frozen until February 8, 1916, when the spores were gradually thawed out and then tested for germination.

TABLE 1

Results of germination tests of spores kept in silo

NUMBER OF TRIALS	SPORE LOT TESTED	MEDIUM IN WHICH TESTS WERE MADE	PERCENTAGE OF GERMINATION
		Sample I	
11	Silo	Distilled water	0
10	Check	Distilled water	25
5	Silo	Modified Cohn's solution	0
5	Check	Modified Cohn's solution	90
6	Silo	Tap water	0
5	Check	Tap water	12.5
		Sample II	
42	Silo	Modified Cohn's solution	0
14	Check	Modified Cohn's solution	90 to 100
10	Silo	Distilled water	0
8	Check	Distilled water	5 to 15
5	Silo	Tap water	0
5	Check	Tap water	15
		Samples III and IV	
18	Silo	Modified Cohn's solution	0
7	Check	Modified Cohn's solution	95 to 100
10	Silo	Sterilized distilled water	0
5	Check	Sterilized distilled water	75 to 95
7	Silo	Tap water	0
3	Check	Tap water	50 to 75
		Sample V	
50	Silo	Modified Cohn's solution	0*
16	Check	Modified Cohn's solution	75 to 95

* One spore germinated.

Sample V. This sample was collected and buried in a silo about 12 feet from the top and 2 feet in from the side on October 4,1915. It was recovered March 16, 1916, thawed out in the laboratory and germination tests were made immediately. The results of the germination tests are summarized in table 1.

The table shows that with but one exception the smut spores did not germinate after having been in the silo. Samples I and II were both tested at the time they were collected, when 68 per cent and 28 per cent, respectively, germinated in water. The single spore which germinated shows a spore may occasionally retain its viability after having been in silage.

The death of the spores may be attributed to a number of possible causes—(1) unfavorable temperatures, (2) gases produced during the changes in the silo, (3) the acids and other chemical substances produced by fermentation, (4) pressure. The effect of each of these factors except the last was tried.

The effect of temperature. The exact changes which accompany the formation of silage from green corn are but imperfectly known. Esten and Mason (5), however, have shown that silage ferments best between 75°F. and 85°F., and that the temperature never rises above 86°F. in properly prepared silage, except in the topmost layers where destructive fermentation occurs. Neidig (8) reports a maximum temperature of 91°F. Such temperatures are not in themselves sufficient to kill corn smut spores, since Stewart (9) found that a temperature of 52°C., for fifteen minutes was necessary to kill the spores when immersed in water. He also found that exposure to dry heat between 105.5°C. and 106°C., for fifteen minutes killed the spores.

In a few tests made by the writer some spores withstood a dry heat of 103°C. for five minutes, but as a rule a temperature of 100°C. for five minutes is sufficient to destroy the germinating powers of the spores.

Freezing temperatures do not seem to injure the spores as they survive our severest winters. Spores, stored in a shed where they were merely sheltered from snow and rain but were exposed to all the rigors of winter, germinated well in spring. Spores that were frozen for short periods of time and then tested for germination appeared to have been stimulated by the cold. The extremes in temperature which the spores encounter in the silo, therefore, can have no influence on their vitality.

The effect of gases. Little is known of the gases produced in silage formation although it is not probable that they would take an active part in the destruction of the smut spores. Carbon dioxide is probably produced in greatest quantity as a result of the fermentative action. A single experiment was made to determine the effects of the gas on the spores. Dry spores were placed in a bottle and carbon dioxide was passed in. The

spores were thus exposed to the gas for ten days, when germination tests were made. The spores were not only unharmed but germinated much more quickly than those of the same lot which were used as checks. At the end of two days no difference was apparent in the germination of the two lots of spores.

The effects of acids. It seems probable that the loss of viability of spores may be due to the chemical substances produced in silage. Considerable quantities of acids are produced rapidly, the maximum amount of each usually being produced within two weeks after the silo is filled. The total acidity of silage, according to Esten and Mason (5), is about 1.0 per cent to 1.5 per cent, the principal acids in order of their importance being lactic, acetic and propionic. In experiments to ascertain the effect of certain of these acids on the germination of smut spores it was found that a concentration of 1 per cent of either acetic or lactic acids or a combination of the two was sufficient to inhibit smut spore germination. However, spores germinated in a diluted sample of normal silage juice.

The above results are significant in that they indicate to some extent what happens to the spores in the silo. The optimum for the germination of spores is reached at some point in the rise of temperature which accompanies the formation of silage. The germination of the spores is, however, inhibited by the presence of the acids. Acetic acid penetrates rapidly and kills plant tissues. Hence, it is not unlikely that the spores, which are exposed to its action for a long time, are killed by it.

Seasonal factors. Fresh spores were collected from time to time and observations made on their germination. The first tests were made in the summer of 1913 and fresh spores germinated very readily in water at room temperature. The tests made in the summer of 1914 were more complete, beginning with the very first smut spores produced in the field. The first germination test of fresh smut spores was made June 24, the last test on October 10. Fifteen distinct tests were made, the results showing conclusively that fresh spores germinate readily in water. In fact, fresh spores germinated much better than did spores from the same lot kept until winter. The average percentage of germination for the entire series was 42.8, the percentages in different hanging drops varying from 0 to 85, a result often obtained when water is used as the medium for germination. This shows that spore germination in water is somewhat capricious, thus probably explaining the conflicting results obtained by various investigators. The germination in sugar solutions and liquid nutrient media, especially modified Cohn's solution is more uniform, 100 per cent of the spores almost always germinating. No difference could be found in the germination in sterilized distilled water, distilled water, tap water or rain water. Incubating the cells at 24°C. to 38°C. did not seem to influence the rate or amount of germination of the smut spores.

Vitality of sporidia

Methods. In the studies of sporidia, pure cultures of the smut were used throughout. Spores were sown in poured plates of beerwort agar. In about two days the spores germinated and the position of the colonies was marked. At the end of four or five days when the colonies were about the size of a pinhead, they were transferred to beerwort agar slants. A number of other nutrient media were also tried, viz.: carrot agar, nitrogen free agar, beef agar, oat agar and a synthetic agar. Of these, beerwort agar was found to be the best, although a good growth was also obtained on carrot agar.

Cultural characters and morphology. When the colonies first appear they are round, raised, convex, opaque, slightly shiny to dull, light cream in color. As the colonies grow older, the edge becomes somewhat lobed and irregular, and the surface becomes convoluted, ridged, or sharply papillate. The color deepens with age, becoming light lavender in old cultures. The consistency is first soft and ropy, then becomes mucilaginous or rubbery; or, when kept moist, butyrous.

The colonies consist of sporidia which are abjointed from the sides and occasionally from the end of the promycelium. These sporidia are of the same nature as those produced beneath the surface in liquid media. The sporidia produced in the air from a liquid culture are small, sharply fusoid and fairly thick walled. They are produced in long chains. Those produced within the liquid or on solid nutrient media are larger than the air conidia, not as thick walled and are somewhat rounded at the ends. They are plumper, contain more oil globules and are not produced in such long chains. The walls are apparently somewhat mucilaginous. In continuous culture the sporidia produce long germ-tubes, the ends of which are densely protoplasmic and which, therefore, can become resting segments possessing all the properties of sporidia. The germ-tubes become much entangled and give the culture its rubbery consistency, while the disintegration of the empty portions of the hyphae give it its mucilaginous character. A smear from such a culture dries almost instantly and becomes brittle. While sporidia produced in culture may not be exactly like those produced in nature, still they must be very similar to those which we imagine are produced in such great abundance in manure.

Thermal relations. A complete understanding of the thermal relations of corn smut sporidia would not only be of value in throwing additional light upon the phenomena of infection, but it would also show more clearly the optimum conditions for the propagation of the fungus. An attempt was therefore made to ascertain the minimum, maximum and optimum temperatures. Both dried and actively vegetating sporidia were tested.

At temperatures ranging from 20°C. to 26°C. the small sporidia bud

profusely in nutrient solutions. As the temperature is increased to above 26°C. the sporidia show a greater tendency to produce long, slender germ-tubes. At 35°C. growth is somewhat inhibited and the cells begin to show an increase in the number of vacuoles. Increasing vacuolation continues with a rise in temperature to 40°C. where growth practically ceases, while at 46°C. the cells are no longer alive.

Attempts to determine the lowest temperature which the sporidia in liquids or on solid nutrient media can endure gave negative results because they withstood the severest cold of the winter (about −28°C.). Alternate freezing and thawing, however, kills moist sporidia. Desiccated sporidia, on the other hand, were not only able to withstand severe freezing but in some cases were not severely injured by alternate freezing and thawing.

When exposed to alternate freezing and thawing, however, there seems to be some injury, as no subsequent growth occurred in two out of three tests. Smears direct from pure cultures were not killed by drying for one day at 21°C. or for fourteen days at a temperature of from 7° to 9°C.

TABLE 2

The effect of temperature on desiccated sporidia of Ustilago Zeæ. All sporidia dried at 21°C.

TEST NUMBER	NUMBER OF TRIALS	NUMBER OF DAYS DRIED	TEMPERATURE TO WHICH EXPOSED	TIME EXPOSED	MEDIUM FOR GERMINATION	RESULTS*
1	1	1	−10°C. to −7°	14 days	Sterilized distilled water	+
2	1	5	43°	15 min.	Sterilized distilled water	+
3	1	6	Alternate freezing and thawing	31 days	2 per cent sugar solution	−
4	1	7	−2°C. to 3°	12 hrs.	Sterilized distilled water	+
5	1	12	45°	15 min.	Sterilized distilled water	+
6	1	16	28.5°C. to 31°	24 hrs.	Sterilized distilled water	+
7	2	16	−7	12 hrs.	Sterilized distilled water	+
8	1	18	54°C. to 55°	15 min.	Sterilized distilled water	−
9	1	19	40°C. to 50°	16 hrs.	Sterilized distilled water	±
10	1	20	−5°C. to 1° alternate freezing and thawing	12 days	Sterilized distilled water	+
11	1	20	−5C°. to 1° alternate freezing and thawing	12 days	Sterilized distilled water	−
12	1	20	28.5°C. to 31°	24 hrs.	Sterilized distilled water	±

* No growth −; grew weakly ±; grew +.

But sporidia dried six days and then exposed to alternate freezing and thawing for thirty-one days were killed.

Sporidial smears direct on glass cover-slips from the pure culture were unaffected by drying for sixteen days at 21°C. and then at 28.5° to 31°C. for one day. Sporidia first placed in water, then dried for twenty days, also withstood the same temperature. Sporidia in smears were not killed by exposure to from 40° to 50°C. for sixteen hours, after drying for nineteen days, but appeared to be killed at 54° to 55°C. for fifteen minutes,

TABLE 3

The effect of desiccation on the vitality of sporidia of Ustilago Zeæ. All tests for germination made at 21°C.

TEST NUMBER	NUMBER OF TRIALS	METHOD OF PREPARATION	NUMBER OF DAYS DRIED	MEDIUM USED FOR GERMINATION	RESULTS*
1	2	Grown in cells in H_2O 2 days. Slip dried	3	Sterilised distilled water	+ +
2	3	Spores germinating in H_2O in cell. Slip dried	3	Sterilised distilled water	+
3	5	In H_2O in cells. Slip dried	1	Sterilised distilled water	+
4	1	Smears made on slips	12	2 per cent sugar solution	+
4	1	Smears made on slips	56	Modified Cohn's solution	+
4	1	Smears made on slips	6 at 21°C. 31 at alternate freezing and thawing	2 per cent sugar solution	−
5	1	In water on slips	12	2 per cent sugar solution	+
5	2	In water on slips	48	Modified Cohn's solution	−
5	1	In water on slips	20 at 21°C. 24 hrs. at 28 5°–31°C.	Sterilised distilled water	±
6	1	Smears on slips	7	2 per cent sugar solution	+
6	1	Smears on slips	51	Modified Cohn's solution	+
7	1	Smears on slips	16 at 21°C. 24 hrs. at 28 5°–31°C.	Sterilised distilled water	+
7	1	Smears on slips	1 day at 21°C. 14 days at 15°–20°C.	Sterilised distilled water	+
8	3	In water on slips	124	Modified Cohn's solution	−

* · · · denotes very abundant germination; + abundant; ± sparse; − no germination.

TABLE 4

Viability of sporidia of Ustilago Zeæ when smeared on a cover slip and desiccated in light and darkness. All germination tests made in sterilized distilled water at a temperature of 21°C.

NUMBER OF TRIALS	CONDITION UNDER WHICH DRIED	DAYS DRIED	RESULTS
2	Light	1	+
2	Dark	1	+
1	Light	1½	+
1	Dark	1½	+
1	Light	6	+
1	Dark	6	+
1	Light	8	+
1	Dark	8	+
1	Light	11	+
1	Dark	86	+
1	Light	87	+
1	Dark	149	+

after drying for eighteen days. The thermal death point of dried sporidia is, therefore, probably about 54° or 55°C.

Moisture relations. In most cases the sporidia were taken directly from the pure cultures on beerwort agar and smeared on sterilized cover-slips. The slips were then placed in sterilized petri dishes and were allowed to dry at room temperature for various lengths of time. In other cases a water suspension was first made of the sporidia and drops of this were then transferred to the slips and allowed to dry as above. When the germination test was made a drop of the desired medium was added and the slips were then mounted on Ward or Van Tieghem cells.

From the results shown in tables 3 and 4 it is apparent that sporidia can withstand long periods of drying without serious injury. Sporidia when taken directly from pure cultures withstood drying for 149 days at room temperature. Not all of these sporidia, however, remained viable. Sporidia first placed in water and then dried seemed to be less resistant to desiccation. Sporidia thus treated grew after drying for twenty days, but not when dried for forty-eight days. The latter result may be somewhat misleading as the number of sporidia in a water drop is much smaller than the number in a smear from pure culture.

There was no noticeable difference between sporidial smears dried in the dark and in the light. Light, therefore, is probably not very injurious to sporidia.

The above results are not in accord with those of Brefeld (3) who found that sporidia were killed when dried five weeks. Nor are they in accord with the statements of Arthur and Stuart (1) who characterize the sporidia

as "short lived" and further add, "These are borne through the air which must be rather moist or the sporidia will be killed by drying."

Chemical relations. Sporidia were placed in various solutions of acetic and lactic acid, in corn silage juice, and in a mixture of various acids in such proportion as to approximate the composition of silage juice. If corn smut spores germinate when placed in a silo, it was thought that by means of tests with various acids commonly produced in silage the fate of the sporidia might be determined. The sporidia in each case were obtained directly from pure cultures on beerwort agar and were transferred to the solution from which hanging drops were then made.

Sporidia apparently can grow in 1 per cent acetic or lactic acid solutions or in a mixture of the two. In the lactic acid the sporidia tend to produce a greater number of germ-tubes which probably indicates that the medium is slightly unfavorable for growth. The sporidia also grow well in expressed silage juice. It seems probable, therefore, that if spores do germinate in the silo, the sporidia may continue to live in the silo for some time. Whether long exposure to the action of the acids would be detrimental or not was not determined.

A more concentrated mixture of acids such as was used in the silage acid test proved to be deleterious to the growth of the sporidia. The sporidia appeared starved and became greatly vacuolated, a condition which probably precedes their death. The results here obtained are not in accord with those obtained when silage juice itself was used, possibly owing to the lack of sugars or other nutrients in the silage acid mixture. The effect of the traces of butyric and propionic acids alone upon the sporidia has not yet been determined.

SUMMARY

1. The infection of corn by *Ustilago Zeæ* (Beckm.) Unger is purely local; no evidence of systemic infection was obtained.

2. When very young plants become infected they are often killed.

3. Injury to the host plant, close planting, very early or very late planting, and growth on rich soil are conducive to heavy smut attacks.

4. Vigorously growing plants, between two and three feet high, are most susceptible to smut attack.

5. The spores of *U. Zeæ* can cause infection either when young or old. Spores germinate readily as soon as mature and retain their viability for several years; infection was obtained by inoculating corn plants with spores five years old.

6. The corn-smut fungus does not lose its virulence quickly when grown on artificial media.

7. The spores of *U. Zeæ*, almost without exception, lost their viability after having been kept in a silo for a few weeks.

8. The factors causing spores to lose their viability in the silo have not been determined definitely; it seems probable that the silage acids, especially acetic, may be the destructive agents.

9. Sporidia were kept in pure culture continuously for three and a half years, at the end of which they remained viable. Inoculation experiments with the same material gave inconclusive results.

10. Sporidia were desiccated for about five months without seriously impairing their vitality.

11. Freezing injures sporidia but little; alternate freezing and thawing, however, is injurious to moist sporidia, less so to desiccated sporidia.

12. The optimum temperature for the budding of sporidia is between 20° and 26°C., the maximum at about 40°C. and the thermal death point near 46°C.

13. Sporidia can germinate and bud in silage juice, but are injured in a solution containing acids in the proportionate concentration in which they occur in silage.

14. The ability of sporidia, as well as spores, to withstand unfavorable conditions is very significant in explaining some of the facts in the parasitism of *U. Zeæ*.

AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF MINNESOTA

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SCLEROTIUM BATATICOLA

The Cause of a Fruit-Rot of Peppers

William H. Martin

During the past few years the writer's attention has been called a number of times to a rot of peppers. Specimens were sent in from various sections of New Jersey with inquiries as to the nature of the trouble. Except for a drying and shrivelling of the epidermis in the more advanced stages, there was little external evidence of the presence of the disease. On breaking the fruit open, however, the interior was found to contain numerous, small, black sclerotia which were also present on the seed. Isolations were made, and in all cases pure cultures of an organism resembling *Sclerotium bataticola* Taub. was secured. The marked similarity of the two organisms led to experiments to establish their pathogenicity as well as to determine if they were identical.

The fungus was isolated by aseptically breaking the diseased fruit; bits of tissue were then picked out with a sterilized needle and transferred to a poured plate of nutrient agar. In most cases pure cultures were obtained at the first planting. Inoculations were made as follows: Healthy pepper fruits were immersed for ten minutes in a 1 to 1000 solution of mercuric chlorid, washed in sterilized, distilled water and placed in a sterilized moist chamber. Attempts to produce the disease by placing bits of the culture media together with sclerotia on the unbroken surface failed. White tufts of mycelium were formed but the fungus seemed unable to penetrate the epidermis. However, inoculations on a cut surface made with a flamed scalpel were uniformly successful. A large number of inoculations were made in this manner and 100 per cent infection resulted. Infection was evident in from four to seven days. The epidermis at the point of infection became blackened and the rot spread throughout the interior. After the fungus became established, its progress was rather rapid; in two weeks the entire interior of the fruits were invaded and numerous sclerotia developed in the tissue and on the seed. Except for a blackening of the epidermis there were no external signs to indicate the presence of the disease. Inoculations were also made on fruit still attached to the plant and in every case the results were positive.

Attempts were made to inoculate the roots and stems of growing plants. The soil was carefully removed from around the roots and an incision was

made with a sterilized scalpel and sclerotia from young cultures were inserted. The soil was then replaced. Roots similarly treated, but not inoculated, were used as checks. Stems were likewise inoculated and the cut surfaces were wrapped with cotton. On the stems and roots the inoculations were not as successful as on the fruits. Where infection did occur the fungus was found just beneath the epidermis or in the pith. Death of the plant or plant part resulted before the mycelium had advanced far from the point of inoculation.

Reisolations were made from both the fruit and stem and the fungus was again grown in pure culture. Inoculations with these reisolated cultures were, in every case, successful.

During the process of the above inoculations, similar inoculations were made on peppers with cultures of *S. bataticola* Taub. isolated from the sweet potato. In every instance they were successful. Not only was this true but the characteristics and growth of the organism were, in all respects, like the pepper Sclerotium.

In order to more fully prove the identity of these two pathogenes, the following comparisons were made: (1) Growth on culture media, (2) cross-inoculations on sweet potato and pepper as well as other hosts, (3) development, measurements and the external and internal appearance of sclerotia.

Growth comparisons were made on corn meal agar, potato agar, bean plugs and nutrient agar. Abundant growth was made and no differences were observed.

For the cross-inoculations three strains of the organism were used. One was isolated from the sweet potato, another, secured from Dr. J. J. Taubenhaus, was likewise isolated from the sweet potato. The third was isolated from peppers. These cultures may be designated 1, 2, 3, respectively. The methods employed in the inoculations were the same as have been previously described. The following inoculations were made:

TABLE 1

Results of inoculations on various hosts with three strains of Sclerotium bataticola

HOST	NUMBER INOCULATED WITH EACH CULTURE	NUMBER OF CHECKS	PER CENT INFECTION
Sweet potato (Ipomœa batatas)	25	10	100
Pepper (Capsicum annuum)	25	10	100
Tomato (Lycopersicum esculentum)	3	2	100
Cucumber (Cucumis sativus)	3	2	100
Apple (Pyrus malus)	3	2	100
Eggplant (Solanum melongena)	1	1	100
Turnip (Brassica campestris)	3	2	100
Red beet (Beta vulgaris)	3	2	0
Parsnip (Pastinaca sativa)	3	2	0
Carrot (Daucus carota)	3	2	0

In those cases where the inoculations were successful, no differences in growth of the organism were evident. The time before infection is evident, varies, however, with different host plants. On pepper, cucumber and tomato from four to seven days is required, while on sweet potato, apple, turnip and eggplant, from three to seven weeks elapses.

The development of sclerotia was followed in Van Tieghem cells. The method employed was to pour enough of a melted nutrient medium on a cover glass to form a thin film. After this had cooled, it was inoculated and inverted over the glass ring. If care is taken in this procedure no contamination results. By this method it is easily possible to trace the development of a single sclerotium under high magnification of the microscope.

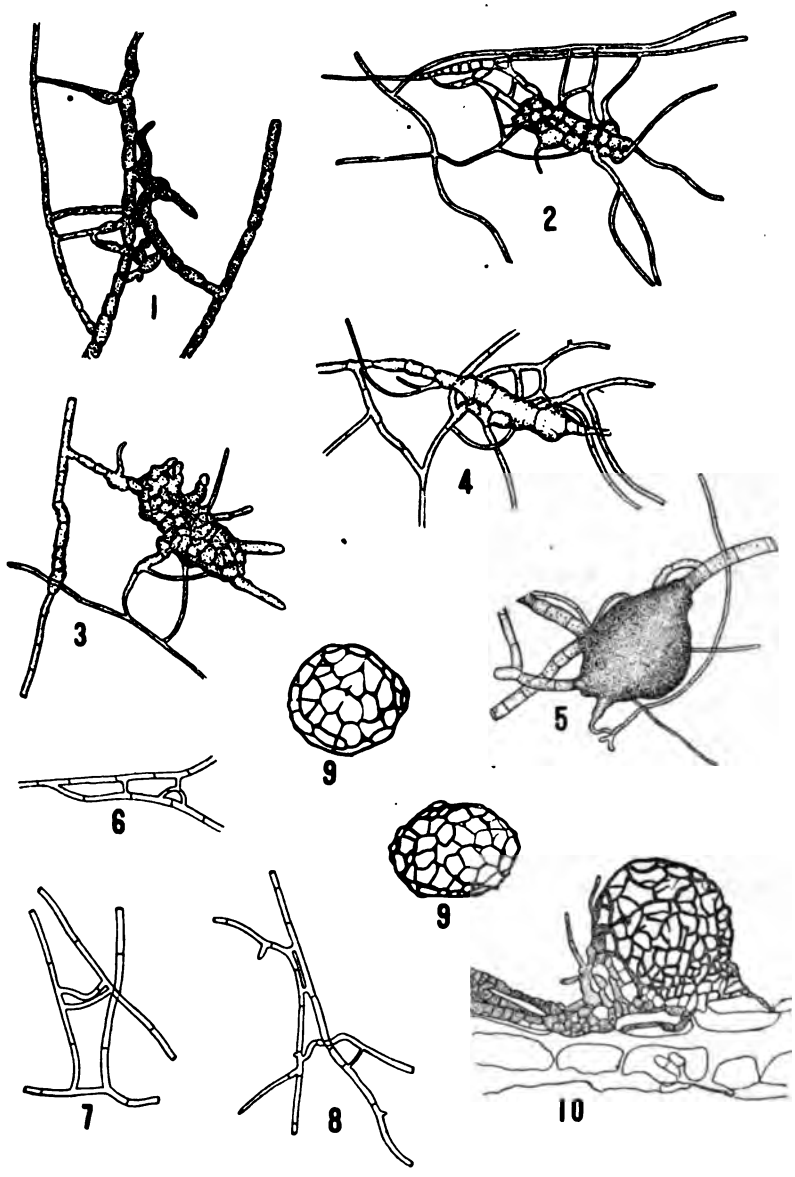
Some cases were noted where sclerotia appeared to have developed from a single mycelial strand; in the majority of cases observed, however, several or a number of strands were involved. Sclerotial development is preceded by the formation of many septa. Short mycelial tubes are formed which connect the strands. These in turn become very much septate, as do the radial hyphae which are formed later (fig. 1). As the sclerotium enlarges the central cells become contorted and form a compact mass, due perhaps to the pressure or resistance of the outer cells (figs. 2, 3, 4). The mature sclerotia are coal-black in color and in most cases are free from any surface irregularities (fig. 5). No differences were observed in the formation or color of sclerotia of the different strains.

In the progress of these studies numerous instances were observed where mycelial branches united; this took place between branches of a single strand as well as between two separate strands (figs. 6, 7, 8). In some few cases, this was the first step in sclerotial development, but usually the mycelium becomes septate and irregular.

Sections of the sclerotia of the different strains showed them to be alike with regard to their internal structure (figs. 9 and 10). In this connection seeds were sectioned to determine if the mycelium penetrated the seed coat, but in none of the cases examined was this observed to be true. The appearance of sclerotia on the seed did not appear to affect germination. Healthy plants were grown from diseased seed. Measurements of the sclerotia gave results identical with those of Taubenhaus.[1]

Attempts to produce a perfect stage failed. In every case, on the germination of the sclerotia new sclerotia were formed.

[1] J. J. Taubenhaus. The black rots of the sweet potato. Phytopath. 3: 159-163; 1913.



SCLEROTIAL DEVELOPMENT OF SCLEROTIUM BATATICOLA

FIGS. 1, 2, 3, 4 and 5. Stages in sclerotial development.
FIGS. 6, 7 and 8. Anastomising of hyphae.
FIG. 9. Sections through mature sclerotia.
FIG. 10. Section of sclerotium on epidermis of pepper fruit.

CONCLUSIONS

The pathogenicity of *Sclerotium* sp. causing a rot of peppers (*Capsicum annum* L.) has been established.

The following facts warrant the conclusion that the sclerotium occurring on peppers is identical with *Sclerotium bataticola* Taub.

a. Cross-inoculations on sweet potato and pepper as well as other hosts gave positive results.

b. With both strains the growth was identical both in culture and on the host.

c. Measurements of the sclerotia are identical.

d. Sclerotial development, color and structure is the same with both strains.

It appears from these studies that the charcoal rot of sweet potatoes (*Ipomœa batata*) is common and widely distributed throughout sections of New Jersey, that *S. bataticola* Taub. can probably persist on several other hosts and that it is the cause of a disease of minor importance of peppers.

LABORATORY OF PLANT PATHOLOGY
NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

A NECTRIA PARASITIC ON NORWAY MAPLE

MEL. T. COOK

During the summer of 1913 the writer's attention was called to an interesting disease on a group of Norway maples growing on the private grounds of one of the residents of Princeton, N. J. The first symptoms of the disease were the wilting of the leaves and dying of branches very similar to the well known symptoms of the chestnut bark blight disease, caused by *Endothia parasitica*. In fact, it was the very striking resemblance to the chestnut blight that attracted the attention of the superintendent of the grounds and led to the writer's being called to make an examination. A further examination of the dead branches showed a still more marked resemblance to the chestnut bark blight; the dead branches had been completely girdled by a canker which showed a blackening and slight sinking of the diseased bark. The older cankers were covered with the orange-colored pustules of the Tubercularia stage of a Nectria and there was abundant evidence that this organism was the cause of the trouble.

The superintendent had been watching the disease for some time and had removed many dead branches and cankers. Fresh wounds were very quickly attacked by the fungus, which made its first growth in the oozing sap, but the careful treatment of these wounds with antiseptics, followed by an application of paint, greatly reduced the number of infections. The breaking of the small lateral twigs from the trunk and larger branches was the most common source of natural infections and most of the cankers had started from wounds of this kind.

An inspection of the trees on the estate and in the immediate vicinity showed two others badly affected. These were destroyed, but the clump previously referred to was left standing for observation and study. During the remainder of 1913 the infected parts were removed as soon as detected and the wounds treated. In the fall of 1913 and the spring of 1914 heavy applications of fertilizers were applied to all the trees and observations continued during the summer of 1914. The disease reappeared and the fungus was always present on the cankers, but was much less severe than in 1913. The fungus was frequently found in the dead bark around old wounds, but in many cases did not appear to be parasitic. About one-third of the trees in this clump showed the fungus in 1914. Another clump of trees on the opposite side of the driveway showed but

very little of the fungus. The clump of trees has been kept under observation during 1915 and 1916, and the disease, although present is much less severe than in 1913. Wounds are frequently infected but the increased vigor of the trees, and the careful removal of diseased branches as soon as detected, has apparently resulted in a great reduction of the disease.

Since 1913 the writer has frequently found the fungus on Norway maples and also on the mulberry, working saprophytically and also apparently as a weak parasite.

The idea that Nectria is parasitic is not new in either Europe or America. Wehmer[1] reports having found it on the healthy stumps of a cut-over thicket of trees and shrubs, especially on *Carpinus* sp. He also found a Diplodia and a Tuberculariа on the young twigs of walnut, sometimes associated and sometimes the Tuberculariа growing alone. He states that the twigs were winter-killed, and that the fungus gained access to the host through the dead parts from which it worked its way into the living tissues. The injured twigs were defoliated and the tree bore very little fruit.

It was also reported by Behrens[2] as attacking *Abies balsamea*. The terminal buds were frequently swollen. This was due to the formation of a layer of cork between the healthy and necrotic tissues. The swellings of the previous year were mostly dead. Some of the green twigs did not develop their buds and some died later. The mycelium hibernated in the dead wood and penetrated the living wood the following season.

The most important American report is by Pollock[3] who found *Nectria coccinea* (Pers.) Fr. causing cankers on yellow birch. These cankers frequently girdle the infected parts. In case of infected twigs hypertrophies were frequently formed. Several American students have reported similar observations to the author.

AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

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PHYTOPATHOLOGICAL NOTES

Notes on Razoumofskya campylopoda. Berries of the false mistletoe, *Razoumofskya campylopoda* (Engelm.) Piper, growing on *Pinus sabiniana*, were collected by Dr. E. P. Meinecke in the San Rafael Mountains, Santa Barbara County, California (Forest Pathology No. 17026), November 12, 1914, and sent to the writers. Seeds from these berries were used November 19, 1914, for inoculating young pine trees in pots as follows: one *Pinus banksiana* Lamb., one *P. bungeana* L., four *P. caribaea* Morelet, four *P. contorta* Loud., two *P. coulteri* Lamb., one *P. densiflora* Lieb. & Zucc., two *P. halapensis* Mill., six *P. mayriana* Sudw., two *P. monophylla* Torr. & Frem., one *P. nigra* Arnold, two *P. parviflora* Lieb. & Zucc., six *P. pinaster* Ait., one *P. pinea* L., one *P. resinosa* Ait., two *P. rigida* Mill., four *P. sabiniana* Dougl., one *P. strobus* L., and two *P. virginiana* Mill. Two trees of *Larix occidentalis* Nutt. and two of *Pseudotsuga taxifolia* (Lam.) Britton were also inoculated. The trees used were from three to six years old. The seeds, enclosed in pulp, were placed chiefly in the axils of the leaves on the younger portions of the shoots, adhering firmly as soon as the pulp dried. Many germinated, but the radicles of only a few succeeded in penetrating the bark of the trees on which they were borne. In six months plants became established on the following species of trees: one *Pinus banksiana*, one *P. bungeana*, one *P. caribaea*, one *P. pinea*, two *P. sabiniana*, and two *P. virginiana*. On *Pinus bungeana* and *P. virginiana*, dense witches-brooms formed around the mistletoe infested region. On the other species spindle-shaped swellings without witches-brooms were usually produced at the point of attack. All these trees except *Pinus sabiniana* are new hosts for this species of mistletoe in this country.

All the trees inoculated successfully produced clusters of mistletoe plants in 1916, none of which produced mature fruits, apparently owing to lack of fertilization. In 1917 mistletoe plants are again developing on all trees except those with dense witches-brooms.

The effect of the mistletoe is to stunt appreciably the growth of all the trees inoculated, as compared to other similar trees of the same species not inoculated. Only one of the trees successfully inoculated has died from this effect after two years' growth, one of *Pinus virginiana* with a witches-broom. In case of trees of the same species inoculated under similar conditions with *Peridermium cerebrum* Peck, and *P. harknessii*

Moore, nearly 50 per cent of the infected trees died inside of two years, indicating that the stem rusts are much more destructive to young pines than the false mistletoes.

The mistletoe is a western species which grows vigorously on eastern species of pines. Since the infected areas on young pines may not be conspicuous during the first season's growth and because of the fact that the aerial parts of the mistletoe plants are annual, and are not usually observed on dormant trees, inspection of nursery stock is not sufficient to insure its freedom from this harmful parasite. Shipments from the Rocky Mountain and Pacific regions to those farther east should be discouraged, as they are likely to carry the mistletoe, even though they may appear clean. Our eastern pines are at present free from mistletoes, and should remain so.

GEO. G. HEDGCOCK AND N. REX HUNT

The production of spores by Alternaria Solani in pure culture. This fungus has been the subject of special study by the author at the University of Wisconsin during the past three years. The scarcity of spore production in pure cultures, which has been noted by previous workers, was at the outset a hindrance to inoculation experiments. Trials with twenty different kinds of media including some tests on the effect of variation of acidity and temperature were made, but the result was always the same, few spores being produced. In February, 1915, experiments were begun in which the moisture content of the medium and the humidity of the air above the culture were varied. This likewise was without results except when the culture was shredded and the mycelium severely wounded. Under such treatment enormous sporulation was always secured (fig. 1, *B*). A few spores were formed when the mycelium alone was cut. The most successful method consists in growing the Alternaria in petri dish culture, on hard potato agar, for ten to twelve days, then, first, shredding the agar to bits (fig. 1, *A*) and stirring to separate and evenly distribute the pieces; second, controlling for twenty-four to forty-eight hours thereafter the moisture relation so that partial drying out is effected without allowing the more exposed surfaces to become desiccated and hard. The latter object is accomplished most readily by removing the lid and exposing the dish to sunlight in a sterilized moist chamber or bell jar. If evaporation is too rapid, occasional atomizing with sterilized water is necessary. Spores were obtained by this method in total darkness in the incubator at 26° C. but closer attention was required.

Over the cut and exposed surfaces of the agar there develops from the old mycelium a network of closely septate, thick-walled hyphae, from which

the conidiophores arise. The initial stimulus for sporulation seems to be largely the result of the wounding of the mycelium in connection with changes in the vapor tension of the air in which it is exposed. After the spores have been rinsed off, a second and even a third crop may be ob-

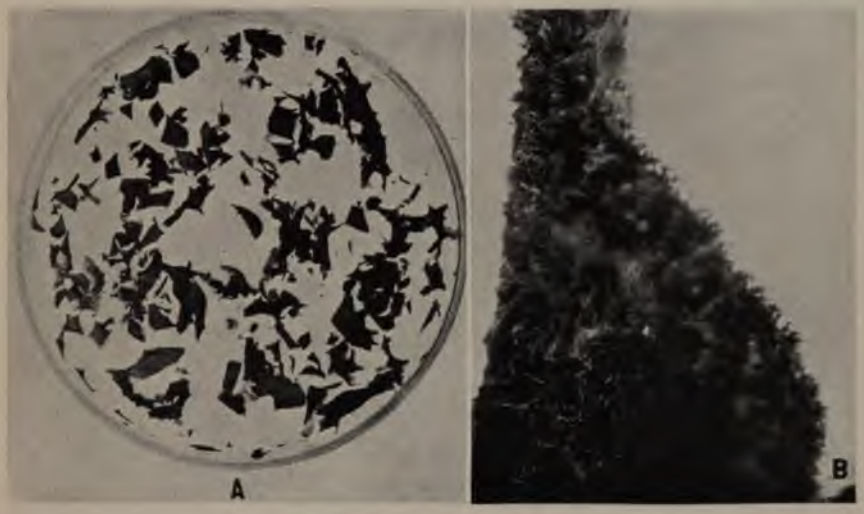

FIG. 1. ALTERNARIA SOLANI

A, Photograph of shredded culture. *B*, Fragment of same magnified × 32 to show abundant sporulation.

tained by moistening the surface and exposing as above. Measurements indicate greater uniformity in size of spores thus produced than on the spots in nature. This method may prove useful with some other fungi which sporulate poorly in culture.

R. D. RANDS

Lightning injury to sugar cane. In a recent note (Phytopath. **7**: 140–142) the lack of reports of lightning injury to any of the Gramineæ was noted. In this connection it may be of interest to record an instance of this kind observed by the writer.

During the past year there was called to our attention a spot in one of the sugar cane fields at the Insular Experiment Station, Rio Piedras, Porto Rico, in which the cane had been completely killed out. The area was nearly circular and approximately a rod in diameter, sharply set off from the surrounding cane, which at the time the first observation was made was at its full height.

In the area itself only a few dead and dry stalks, some broken off 2 or 3 feet above the ground level and some prostrate, remained. There had been no growth of new shoots, and examination showed that the underground portions of the stools were likewise dead. There was a growth of weeds present but entirely of herbaceous annuals, which could have been seeded from the margin of the area. No signs of insects, rodents, or fungi were found either above or below ground, not even of the common saprophytic forms so common on dead and dying canes, of which some eighty are known. The surrounding cane was likewise entirely free of insects or fungous diseases and normal in all respects.

FIG. 1. LIGHTNING INJURY TO SUGAR CANE

Charred bits of cane trash were finally discovered, which fact, combined with the other observations, indicated that lightning was the cause. No other source of fire could have destroyed green cane so completely, especially the underground portions of the stools. The burning over of cane fields is a very common practice in Porto Rico, and fields so treated have been observed in innumerable instances, so that the possibility of an ordinary surface fire through a carelessly dropped match or other source having been the cause need not be considered.

Occurrences of this kind are apparently rare.

JOHN A. STEVENSON

Phytophthora infestans, causing damping-off of tomatoes. Phytophthora infestans has been recorded as occurring on tomatoes by both American and European pathologists. So far as literature on the subject is available to the writer, no one appears to have noted this organism as causing damping-off of young tomato plants. The following brief account of a severe outbreak of damping-off of tomatoes caused by *Phytophthora infestans* may be of interest therefore to pathologists.

In June, 1916, a number of young diseased tomato plants were received by the Ontario Agricultural College from J. W. Noble, Essex, Ontario. In the letter accompanying the plants Mr. Noble stated that thousands of late tomato plants in that district had been destroyed. The affected plants were first observed shortly after the tomatoes were set in the field.

A glance at the plants revealed brown lesions and constrictions on the stems near the surface of the ground. Many of the plants showing these symptoms had fallen over, due to the collapse of the stems at this point. On examining the stems with a hand lens a white fungous growth was clearly seen on the lesions. This when examined under the microscope proved to be the conidiophores and conidia of *Phytophthora infestans* (Mont.) deBary. After this all the plants were examined very carefully, and on some of them the same fungus was found, apparently causing a blighting of the leaves; but the chief damage done to all of them was by the destruction of the stem near the surface of the ground. The falling over of the plants by hundreds in the field was what first brought the disease to the attention of the growers.

Some idea of the severity of this outbreak of damping-off of tomato plants may be had by considering the fact that out of 288,175 tomato plants supplied by the Heinz Pickle Company, Leamington, Ontario, to Pelee Island growers, only 45,000 reached maturity. At least 50 per cent of the plants that did not survive succumbed to damping-off due to *Phytophthora infestans.*

It is interesting to note that weather conditions during June were exceptionally favorable to the spread and development of *Phytophthora infestans,* the rainfall being much above the average for June, and the temperature relatively low.

J. E. HOWITT

State and National quarantines against the white pine blister rust. The following table shows the State and National quarantine action taken to date against the white pine blister rust. The action is so varied in character that it seems necessary to present it in this form. Similar action is under consideration in a number of other states.

STATE	WHITE PINES	RIBES, GROSSULARIA	DATE	QUARANTINED AREA
Canada	All	None	November 14, 1914	All foreign countries
United States	P. strobus, monticola, lambertiana, cembra	None	September 16, 1912	Great Britain, France, Belgium, Holland, Denmark, Norway, Sweden, Russia, Germany, Austria, Italy, Switzerland
	All	None	May 21, 1913	Europe and Asia
	All	All	March 16, 1916	Canada and Newfoundland
	All	All	June 1, 1917	All points east of, and including the states of Minnesota, Iowa, Missouri, Arkansas, Louisiana
	All	R. nigrum	June 1, 1917	States of New England and New York
	None	All	June 1, 1917	Europe and Asia
California	All	All	February, 1917	East of Mississippi River
Delaware	All	All	March 2, 1917	All points outside state
Idaho	All	All	March 1, 1916	New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania
Indiana	All	All	March 13, 1917	All points outside state
Kansas	All	All	March 10, 1917	All points outside state
Massachusetts	All	None	June 1, 1912	Europe
Michigan	All	All	March 19, 1917	All points outside state
Minnesota	All	None	April 30, 1917	Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Ohio, Wisconsin
Montana	All	All	July 17, 1916	New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania
Nevada	All pines	All	March 10, 1917	East of Mississippi River and Minnesota; all foreign countries
New Jersey	All	None	April 16, 1917	Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, Pennsylvania, New York, Minnesota, Wisconsin

STATE	WHITE PINES	RIBES, GROSSULARIA	DATE	QUARANTINED AREA
New York.....	All	None	March 24, 1917	Ohio, Indiana, Minnesota, Wisconsin, Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, Pennsylvania, Illinois, New Jersey
Ohio.....	P. strobus, monticola, lambertiana, cembra, excelsa, flexilis	None	February 21, 1917	All points outside state
Oregon.....	All	All	July 24, 1916	East of Mississippi River; all foreign countries
Pennsylvania...	All	None	March 12, 1917	All points outside state
South Dakota...	All	All	April 3, 1917	All points outside state
West Virginia...	All	All	April 18, 1917	All points outside state
Wisconsin.....	P. strobus, monticola, lambertiana, cembra, excelsa	None	June 1, 1916	All points outside state

PERLEY SPAULDING AND ROY G. PIERCE

Personals. Arthur S. Rhodes, assistant in forest botany at the New York State College of Forestry, Syracuse, New York, has been appointed assistant in the Office of Forest Pathology, Bureau of Plant Industry.

Miss Ruby J. Tiller, scientific assistant in the Office of Forest Pathology, Bureau of Plant Industry, has resigned her position to become the wife of Prof. S. F. Acree of the University of Wisconsin.

Prof. L. H. Pennington of the New York State College of Forestry, Syracuse, New York, has accepted a temporary position as expert with the Office of Forest Pathology, Bureau of Plant Industry. Doctor Pennington will have charge of the season's work on white pine blister rust eradication in the state of Michigan.

Mr. G. H. Godfrey, of Iowa State College, was appointed scientific assistant in Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, effective June 8. Mr. Godfrey was formerly scientific assistant in Cereal Disease Investigations, but during the past year was granted leave of absence to engage in post-graduate study.

LITERATURE ON PLANT DISEASES

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

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[PHYTOPATHOLOGY, for June, 1917 (7: 155–234) was issued June 2, 1917.]

PHYTOPATHOLOGY

VOLUME VII NUMBER 5

OCTOBER, 1917

ALTERNARIA ON DATURA AND POTATO

R. D. RANDS

WITH FOUR FIGURES IN THE TEXT

During the progress of studies on the early blight of potato caused by *Alternaria solani* (E. & M.) J. & G., an investigation has been made of the various host relations attributed to this fungus. Throughout the literature the early blight organism is claimed to be the cause of the leaf-spot of Jimson weed (*Datura*, various species). One author (Chester, 1892) goes so far as to state that this was probably the original host, the fungus going from it to the potato and tomato. The object of this paper is to present some comparative studies of early blight and the Datura leaf-spot in order to clear the apparent misunderstanding of the relationship between these two diseases.

The Datura leaf-spot has been under the observation of the author in the University Pharmaceutical Garden, Madison, Wisconsin, during the summers of 1915 and 1916. The spots show a zonation similar to that of early blight but they are straw colored rather than deeply stained (fig. 1). They first appear on the lower more shaded leaves. Under favorable conditions the disease spreads gradually upwards when finally, in late autumn, the seed pods often develop dark sunken lesions (fig. 2). When a leaf becomes weakened by a number of enlarged spots it is usually shed from the plant.

The disease was noted on the following species and varieties growing in the Pharmaceutical Garden September 15, 1915:[1]

Datura tatula Linn., *D. tatula inermis*, *D. stramonium* Linn., *D. stramonium inermis*, *D. stramonium giganteum*, *D. inermis* Jacq., *D. fastuosa* Linn., *D. ferox* Linn., *D. laevis*, *D. quercifolia* H. B. K., *D. leichardtii* F. Muell., *D. metel* Linn.(?).

[1] The species and varieties are listed in the form in which they were found on the garden labels. Since the seed was originally of German origin the names are presumably those in common usage in the foreign seed trade.

The pod blight was especially conspicuous on *D. tatula inermis*, *stramonium inermis* and *fastuosa*, while the leaf-spot occurred on all to a greater or less extent. *Datura stramonium giganteum* showed greater resistance than the others.

ECONOMIC IMPORTANCE OF THE DISEASE

As is well known, the Daturæ furnish one of the sources of the drug atropin or daturin which is obtained from the leaves. The leaves are picked in late September sufficiently early to avoid frost. It is evident that anything which tends, particularly in the latter part of the season, to reduce the leaf area seriously interferes with its economic use. Estimates made in September 1915, placed the average for all varieties at 40 to 50 per cent of the total leaf surface destroyed. The dry season of 1916 prevented much appearance of the disease prior to the rains of September. But even after that, favorable weather continuing, a loss of 10 to 15 per cent in leaf area occurred.

HISTORY OF THE DISEASE AND ITS CONFUSION WITH EARLY BLIGHT

The first reference inferring that a relationship exists between this disease and early blight is that by Cooke (1883). He describes, as though they were identical, the fungus from Datura and that from the tomato as *Macrosporium solani* Cooke. He was apparently unaware that the same binomial had been applied by Ellis and Martin to a similar fungus on the potato the year before. Saccardo (1886) lists Cooke's fungus as *Macrosporium cookei* Sacc. Later Ellis (see Jones 1893) affirms that this is the same fungus which he and Martin described. Thus the confusion seems to have originated. It is shown by the following references which are apparently concerned with this Datura leaf-spot. Kellerman (1885) from Manhattan, Kansas, Halsted (1893) from New Jersey, Briosi and Cavara (1892) and Ferraris (1913) from Italy, Stevens (1896) from Ohio, Underwood and Earle (1897) and Atkinson (1897) from Alabama, Jones and Grout (1897) and Orton (1899) fom Vermont. So far as the literature reveals the above determinations were based entirely on morphological evidence and on the similarity of the disease to early blight of potato and not on inoculation work with the causal organism.

In order to determine whether the leaf-spot as found at Madison is the same as that reported elsewhere, exsiccatae labelled *Macrosporium sol.* E. & M. on Datura, were examined and compared with typical material collected here. The macroscopic appearance of the spots of the exsiccatae of Seymour and Earle (No. 340) and other collections from

FIG. 1. LEAF-SPOT OF DATURA TATULA CAUSED BY ALTERNARIA CRASSA
Photographed September 20, 1915

Florida, Mississippi, Illinois, Kansas, and North Carolina is identical with the Madison material. During the summer of 1916 letters of inquiry and request for fresh material were sent to a number of plant pathologists in different parts of the United States. Specimens received from H. H. Whetzel, Ithaca, New York; B. B. Higgins, Experiment, Georgia; J. A. Elliott, Newark, Delaware; and C. D. Learn, Stillwater, Oklahoma, confirmed the above conclusions concerning the identity and wide distribution of this disease. The material represented only the two common species, *Datura tatula* and *D. stramonium*. Pure cultures from single spores of

FIG. 2. POD BLIGHT ON DATURA STRAMONIUM VAR. GIGANTEUM, CAUSED BY ALTERNARIA CRASSA

an Alternaria were obtained from each lot which, upon comparison and subsequent pathogenicity tests, proved to be identical and the cause of the disease. Reciprocal cross-inoculations were then made with this fungus and *Alternaria solani* from potato on their respective hosts.

INOCULATION EXPERIMENTS WITH ALTERNARIA SOLANI

In table 1 is shown the results of greenhouse inoculations where *A. solani* isolated from potato was comparatively tested on two of its known hosts and on Jimson weed. Besides the introduction of spores or mycelium into needle pricks, the following methods of inoculation were used: (1) Drop of spore suspension in water on flat portion of leaf inclosed beneath an ordinary round cover slip for twenty-four hours. (2) Leaves

atomized with a spore suspension in water and for forty-eight hours kept moist by a fine spray from a nozzle. In most cases reisolations made from the infected plants were successful.

On September 4, 1916, further inoculations with spores of *Alternaria solani* were made on mature potato and Datura plants growing in the field. Several leaves were atomized with a heavy spore suspension, while with others spores were introduced into punctures. Successful reisolations of the fungus was secured from both atomized and needle punctured leaves on the potato. In one instance tissue plantings from the browned needle punctures on *Datura stramonium* five weeks after inoculation gave the fungus. The results corroborate the green house tests and show that, in no case, was *A. solani* able to form spots on even the old and weakened leaves of Jimson weed. However *in toto* fixations showed that penetration and incipient infection occurred in many cases. But the fungus seemed unable to establish itself and bring about enlargement of the spot.

INOCULATIONS WITH THE FUNGUS FROM DATURA

The Madison culture of the parasite from *Datura stramonium* was tested comparatively first in the greenhouse, and later in the field on Jimson weed, potato, tomato, and on *Solanum nigrum*, the common black nightshade. Several methods of inoculation in which mycelium or spores were placed in needle pricks and the spores atomized upon the surface were used. Most of the tests were carried out at the same time and under the same conditions as those already reported with *Alternaria solani* from potato. These experiments may be briefly summarized as follows: On the Jimson weeds including *Datura stramonium*, *D. inermis*, and *D. tatula*, typical spots 3 to 10 mm. in diameter invariably resulted after two weeks. On potato, tomato and *Solanum nigrum*, incipient infections in the form of tiny brown specks often occurred. They were less abundant on the nightshade and vigorous leaves of tomato. These spots in no case enlarged, even after the leaves were yellowing and dying. The fungus was reisolated readily from the Datura leaves but only in a few cases could it be obtained from the incipient spots on the other plants. The needle punctures on the latter were in most cases entirely healed after eight days. Therefore, it appears that we have here an Alternaria which, though bearing much superficial resemblance to *Alternaria solani*, is nevertheless distinct in its host relationship. In no instance has there been observed any crossing over to the potato or other hosts of *A. solani* tested, and also no crossing of the potato fungus to Jimson weed. The above conclusion is further confirmed by a comparison of the two fungi in other particulars.

TABLE 1

Greenhouse inoculations with Alternaria solani, Madison, Wisconsin, 1916

DATE	INOCULUM	PLANTS INOCULATED, CONDITION, ETC.	METHOD OF INOCULATION	RESULTS
February 19	Mycelium	Potato, 10 in. high; vigorous	5 leaves punctured (mycelium + cover slip)	February 23. Dark spots 2 to 4 mm. diam.
			3 leaves with No. 1	February 23. Specks 1 to 2 mm. diam. March 3. All have well developed dark spots 5 to 15 mm. diam. some sporulation
		Datura stramonium, 4 in. high; very vigorous	As with potato	February 23. No evidence of infection March 3. Two punctures straw colored spots infection doubtful; no results under cover slips
March 20	Spores	Potato, 6 to 8 in. high; very vigorous; 11 inoculations	No. 1	March 22. Black dots beneath most of covers April 10. Spots 1 to 3 mm. diam.
		Datura inermis, 2 plants 6 in. high; vigorous; 20 inoculations	No. 1	March 22. No change April 10. No spots developed
		Potato, 1 plant 12 in. high; vigorous	No. 2	April 20. Minute spots on every leaf; wet continuously
April 13	Spores	Tomato, 1 plant, vigorous	No. 2	April 20. Few spots; not continuously wet
		Datura stramonium, 1 plant, vigorous	No. 2	April 20. No evidence of infection; continuously wet

May 7	Spores	Potato, 1 plant 14 in. high; vigorous	No. 2	May 14. Many spots 1 to 3 mm. diam.
		Tomato, 2 plants 16 in. high; vigorous	No. 2	May 14. Spots abundant on lower leaves
		Datura stramonium, 2 ft. high; vigorous	No. 2	May 14. Many specks on lower leaves but no subsequent enlargement

TABLE 2

Field inoculations with Alternaria solani on potato and Datura

PLANT TESTED	SIZE AND CONDITION OF PLANT	RESULT AFTER TWO WEEKS		RESULT AFTER FIVE WEEKS	
		Atomised leaves	Needle punctured leaves	Atomised leaves	Needle punctured leaves
Potato	Young upper leaves; vigorous	Many spots 3 to 5 mm. diam. coalescing	100% infection; spots 3 to 6 mm. diam.	Leaves dying from abundance of spots	
Datura stramonium	2 to 3 ft. high; weakened by drought and red spider	Peppered with spots 0.5 to 1 mm. diam.	Faint browning of margins of punctures	No change	No change
Datura tatula	2 to 3 ft. high; weakened by drought and red spider	Peppered with spots 0.5 to 1 mm. diam.	Faint browning of margins of punctures	No change	No change
Datura inermis	2 to 5 ft. high; fairly vigorous	Few specks found	Faint browning of margins of punctures	No change	No change

COMPARISON OF THE FUNGUS FROM DATURA WITH ALTERNARIA SOLANI

On hard potato agar the colony of Alternaria from Datura is at first of a light olivacious color with faint pinkish margins; later the entire growth becomes a grayish white while from the lower surface often blackish mycelium can be seen through the agar. With *A. solani* the grayish or brownish colony produces a deep pinkish to yellowish pigmentation of the agar which often extends in advance of the mycelium (fig. 3). This furnishes at once a distinct physiological basis for the differentiation of the two fungi. Morphologically they are similar in many respects. One of the most prominent characters which distinguishes the Datura fungus from *A. solani* is found in the terminal prolongation or beak of the spore. In the former the beak is coarser, more elongated, rarely has any pronounced tapering to the tip, and is never forked or divided (fig. 4). Often as high as 75 per cent of the spores of *A. solani* both from cultures and from spots, have been observed to possess forked or variously divided beaks, the subdivisions tapering more or less to the tip. One hundred spores from typical early blight spots from potato gave a range in size of 120 to 296 by 12 to 20 μ and an average of 200 by 17 μ. With the Datura fungus the range in size was 128 to 448 by 16 to 40 μ with an average of 261 by 23 μ. The latter it is seen has spores considerably larger in both dimensions than *A. solani*. However, the difference is not such as to make spore measurements reliable when not supplemented by the other distinguishing characters mentioned.

IDENTITY OF THE DATURA PARASITE

The fungus has been referred to as an Alternaria. This was, however, merely on account of its great similarity to *Alternaria solani*. As in the case of the latter, cultures, on oatmeal agar occasionally develop spores in catenulate pairs (fig. 4). According to the present delimitations of the genera Alternaria and Macrosporium this catenulation of spores makes the fungus an Alternaria. However, it is realized that the conditions under which spore pairs are formed are possibly abnormal and it is doubtful if the fungus behaves similarly under natural conditions on its host. At any rate the examination of many spots has failed to reveal it. Thus the taxonomic position of the fungus is problematical. Until these two genera are more clearly defined it will be called provisionally an Alternaria.

To what species the fungus should be referred was a matter of doubt for some time. A careful search of the literature failed to reveal a description of a species of Alternaria on Datura. One Macrosporium, *M. datura* Faut. (Lambotte and Fautrey 1894), is described as occurring on

Datura stramonium on the sterile spots of an undetermined species of Sphaeropsidales

The spore measurements (150 to 190 by 18 to 20 μ) fall within those given in this paper and the long slender "pedicel" (referring to the terminal beak) also is characteristic of the fungus under consideration. While it is probable that the two may be identical the description is entirely too brief for one to be certain. The question was referred to Dr. J. J. Davis. After looking over the various fungi described for Datura, he expressed the belief that this Alternaria (or Macrosporium), in an immature condition, was probably the same which Saccardo (1877 *b*) describes under

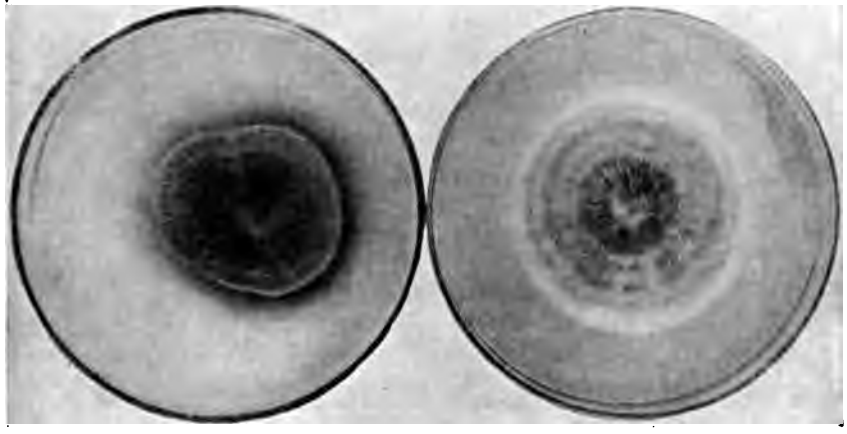

FIG. 3. SEVEN-DAYS-OLD CULTURES OF ALTERNARIA SOLANI (LEFT) AND ALTERNARIA CRASSA (RIGHT)

The colony in the former is light brownish and produces a pinkish discoloration of the agar (hard potato) often extending in advance of the mycelium; the colony of the latter is grayish white and there is no discoloration of the medium.

the name *Cercospora crassa* Sacc. sp. n. He also found that later Peck (1882) describes under the name *Cercospora daturæ* n. sp. from New York State what seems to be the same. The fungus corresponds with Peck's description in all essentials except that the spore measurements (50 to 75 by 12 μ) fall considerably short. The type specimens collected by Peck have been examined and the appearance of the spot leaves little doubt of their identity with the Madison material. From Saccardo's original illustration (1877 a) and the description in Michelia where the spore measurements are given as 100 to 150 by 15 to 18 μ, there is every evidence that he was dealing with immature material of this same fungus. In

order to relieve all doubt, however, a type specimen distributed in Mycotheca Italica No. 996 Padova, August 1901 was examined.[2]

This well-preserved specimen of a spotted leaf of *Datura stramonium* collected by Saccardo was s milar in almost every respect to specimens from Madison and from various other parts of this country. Though the spots are small, their characteristic appearance leaves no doubt of the identity of the two diseases. Furthermore, some spores were obtained from the specimen and they add still further confirmation. As the pres-

FIG. 4. COMPARISON OF SPORE CHARACTERS OF ALTERNARIA CRASSA AND ALTERNARIA SOLANI

In the group of A. crassa (to the left) the first two are typical spores from leaf-spot on Datura stramonium; the third represents a case of catenulation on scalulated potato agar. To the right, typical spores of A. solani showing variation in size and form; these are uniformly smaller than A. crassa and frequently have the beaks branched. × 200.

ence of longitudinal septa in long pointed single spores of Alternaria or Macrosporium is the important character which separates them from Cercospora, it may be readily understood how immature spores of the former might be mistaken for the latter. And Saccardo observed a longitudinal septum, as is shown by his illustration, but he calls it a false one, "septulo spurio." Since it is true that the longitudinal partitions in Alternaria are generally absent until the spores attain full size, it seems probable, as Dr. Davis suggests, that Saccardo's conception was obtained from immature material.

[2] The writer is indebted to Dr. A. B. Stout of the New York Botanical Garden for assistance in making this examination.

Cercospora crassa is listed by Tassi (1906) at the University of Senna, Italy, and also by Ferraris (1910 who gives as its distribution northern Italy, Switzerland, and Germany. The latter describes in addition the following which he considers forms of *Cercospora crassa:* (1) *lunariæ*, on *Lunaria biennis;* (2) *ibiridis*, on *Iberis umbellata;* (3) *solani-nigri*, on *Solanum nigrum.*

These are recorded from Italy alone. The inoculations already described show that *Solanum nigrum* cannot act as a host for this fungus, thus throwing some doubt on the validity of the form reported for this plant. No tests were made on the other plants.

In order to avoid future confusion the probable synonomy of the fungus and a description of the new combination are here given:

Alternaria crassa (Sacc.) n. comb.[3]

Cercospora crassa Sacc. Michelia **1**: 88. 1877.
Cercospora daturæ Peck. Rept. New York State Museum **35**: 140. 1882.
Macrosporium solani Cooke (in part). Grevillea **12**: 32. 1883.
Macrosporium daturæ Fautrey. Rev. Myc. **16**: 76. 1894.
Alternaria solani (E. & M.) Jones & Grout (in part). Bul. Torr. Bot. Club, **24**: 257. 1897.

Conidiophores light brown, erect or ascending, somewhat irregular, septate, generally 2 to 3 cespitose, 70 to 90 by 9 to 10 μ; conidia light brown, obclavate, with very long, septate, filiform, terminal beak, generally much exceeding in length the body of the spore, total dimensions 128 to 448 by 16 to 40 μ, average 261 by 23 μ; body of spore 56 to 140 μ in length, 7 to 9 transverse septa, 1 to 3 longitudinal septa in mature spores (sometimes absent); colony, in potato agar culture, light grayish, cottony, no pigmentation of the medium.

Destructive, causing a leaf-spot and pod blight of Jimson weed (*Datura*, various species).

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

[3] Inasmuch as the fungus in nature produces spores singly, and catenulate spores have been observed only in culture, some authors may prefer the combination *Macrosporium crassa.*

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SUSCEPTIBILITY OF NON-CITRUS PLANTS TO BACTERIUM CITRI

R. A. JEHLE

WITH THREE FIGURES IN THE TEXT

Various non-citrus plants have been inoculated with pure tested cultures of *Bacterium Citri* Hasse in order to determine their susceptibility to the citrus canker disease. Special attention has been given to related species belonging to the Rue family (Rutaceae). The plants are growing directly in the soil in a securely screened inclosure. No results have been obtained from any of these experiments excepting in the case of *Murraya exotica* (Orange Jessamine) and *Zanthoxylum fagara* (L.) Sarg. (Wild-lime).

In the case of *Murraya exotica* a few watery swellings developed around some of the needle-prick inoculations in the leaves and the canker organism was recovered from the interior of one of the swellings a few weeks later. Inoculations were also made by spraying the twigs and leaves with a suspension of canker bacteria, but the results of these latter tests were negative.

Many inoculations were made on the twigs of all sizes and on the leaves of *Zanthoxylum fagara*. On the leaves watery swellings sometimes surrounded by a yellow halo developed around most of the needle-pricks into which canker bacteria were introduced (fig. 2, *A*). These swellings never burst open, but *Bacterium Citri* was recovered from the interior three months after inoculation. On the twigs a total of about one hundred needle-prick inoculations were made. In every case watery swellings developed in about ten days. These swellings on the twigs continued to increase in size and finally longitudinal splitting of the bark took place exposing a mass of corky cells much resembling canker infections on the twigs of *Citrus trifoliata* (figs. 1 and 3). *Bacterium Citri* was repeatedly recovered from these swellings, which produced typical canker on grapefruit seedlings (fig. 3, *D* and *E*). On *Zanthoxylum fagara* twigs the same organisms produced infections identical with those from which they were isolated. Check inoculations were made by pricking the tissues with a sterilized needle, but the wounds soon healed over (fig. 1, *C*).

Twigs of *Zanthoxylum fagara* were inoculated by spraying a suspension of *B. Citri* in distilled water on the uninjured surface with an atomizer. These twigs were covered for several days with a lamp-chimney plugged

FIG. 1. RESULT OF NEEDLE-PRICK INOCULATIONS WITH BACTERIUM CITRI IN TWIGS OF ZANTHOXYLUM FAGARA

A. Two infections on a young twig of *Zanthoxylum fagara* produced by introducing [illegible] tissues with a sterilized needle. × 3. The lower lesion [illegible] twig. Inoculated November 20, 1916. Photograph made February 27, 1917.

B. Two infections on a young twig of *Zanthoxylum fagara* produced by introducing [illegible] tissues with a sterilized needle. × 3. Inoculated November [illegible], 1916. Photographed January 27, 1917.

C. Young twig of *Zanthoxylum fagara* into which no organisms were [illegible]. × 3. Inoculated November 20, 1916. Photographed January 27, 1917.

FIG. 2. LESIONS FROM NEEDLE-PRICK INOCULATIONS ON LEAFLET AND TWIG OF ZANTHOXYLUM FAGARA

A. Two lesions on a leaflet of *Zanthoxylum fagara* produced by introducing *Bacterium Citri* into the tissues with a sterilized needle. × 3. Inoculated May 21, 1917. Photographed July 17, 1917.

B. Five infections on a very young twig of *Zanthoxylum fagara* produced by introducing *Bacterium Citri* into the tissues with a sterilized needle. × 3. Inoculated May 21, 1917. Photograph made July 17, 1917.

FIG. 3

with cotton at the ends. Other twigs were treated in exactly the same manner excepting that sterilized water containing no *B. Citri* was used in the atomizer. No swellings developed on the twigs sprayed only with sterilized water but five cankers developed on the twigs sprayed with *B. Citri*. The five cankers which developed as a result of the atomizer inoculations were almost identical in appearance. They very much resembled young citrus canker infections on the twigs of *Citrus trifoliata*, but differed from the latter in having a distinct transverse split across the center. In one instance the development of one of these cankers was observed for several months. When the infection was found it was a small dark green watery swelling with a small transverse split in the bark across the center. The swelling continued to increase in size, and the transverse split in the bark became more conspicuous. When the canker had reached about three millimeters in diameter, longitudinal splits began to appear in the bark at the outer ends of the transverse split. The cracks exposed a corky mass of cankerous tissue similar to that on other hosts. The largest canker reached a diameter of five millimeters seven months after inoculation. It was surrounded by an oily zone, indicating that the bacteria were still alive and active in the interior. One of the smallest cankers was removed from the tree (fig. 3, *C*) and isolations were made from the interior. The bacteria recovered from this canker did not differ from other strains of *Bacterium Citri*. They produced typical citrus canker on grapefruit seedlings and infections on *Zanthoxylum fagara* identical with infections produced by other cultures of *Bacterium Citri*.

A grapefruit seedling badly infected with citrus canker was set so that its branches interlocked with those of *Zanthoxylum fagara*. Infections identical with those resulting from inoculations with *B. Citri* have developed on uninoculated twigs and leaves of *Zanthoxylum fagara* near to the infections on the grapefruit seedling. These infections undoubtedly resulted from *B. Citri* washed by rains from the infected grapefruit tree to the twigs and leaves of *Zanthoxylum fagara*.

FIG. 3. RESULT OF INOCULATIONS WITH BACTERIUM CITRI ON THREE DIFFERENT HOSTS

A. Result of an atomizer inoculation with *Bacterium Citri* on a twig and thorn of *Citrus trifoliata*. × 3.

B and *C*. Result of atomizer inoculations on twigs of *Zanthoxylum fagara*. × 3. Both twigs inoculated November 17, 1916.

B, photographed July 18, 1917; *C*, photographed February 14, 1917. Isolations were made from *C* on February 15, 1917 and pure cultures of *Bacterium Citri* were recovered.

D and *E*. Result of needle-prick inoculations on twigs of grapefruit with organisms recovered from interior of canker on a twig of *Zanthoxylum fagara*. × 3.

Although lesions have been occasionally noted on the twigs of *Zanthoxylum fagara* in Dade County hammocks, they have been carefully examined and no evidence has been secured indicating an abundance of natural infestations of this plant with the bacteria of citrus canker.

FLORIDA STATE PLANT BOARD
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SOME DISEASES OF ECONOMIC PLANTS IN PORTO RICO

L. E. Miles

With Three Figures in the Text

ISARIOPSIS ON THE BEAN

The Isariopsis leaf-spot of the cultivated bean is a disease which occurs on both the foliage and pods of the bean plant, often resulting in considerable damage to the crop, due to the lessening of the active leaf surface or sometimes to the partial or complete premature defoliation. The disease has not been considered of any great significance commercially, but according to Halstead[1] it is much more common in the United States than is usually supposed, and to it should probably be ascribed much of the trouble formerly attributed to other fungi. It is easily confounded with the leaf-blotch of the bean (Cercospora). The chief points of difference between these two diseases will be considered later in this paper.

The fungus causing the disease, *Isariopsis griseola*, was first described by Saccardo in 1877, as occurring in northern Italy and southern Austria. Briosi and Cavara also report it as occurring in Italy, and it is represented in their exsiccati by a specimen collected near Pavia in 1888. In the exsiccati of Rabenhorst, Winter and Pazschke a specimen is found collected near Zurich in Helvetia. Rabenhorst reports the disease as occurring also in Belgium, Poland and Argentina. It is represented in the collection of North American Fungi of Ellis and Everhart by a specimen collected by J. B. Ellis at Newfield, New Jersey in 1889. In the Fungi Columbiani of Ellis and Everhart, as continued by E. Bartholomew, a specimen is found collected by C. L. Shear at Takoma Park, Maryland, in 1906. Halstead of New Jersey in 1901 reported it as occurring in that state. F. L. Stevens of the University of Illinois found it in abundance on beans in the garden of the Experiment Station of Porto Rico in 1912 and 1913. It is also represented in the collection of J. A. Stevenson by a specimen collected at Rio Piedras, Porto Rico, in 1916. The writer has examined specimens collected by J. L. Sheldon at the following places in West Virginia: Morgantown, 1904; Martinsburg, 1905; Sink's Grove and Bull Run in 1906. The Plant Disease Survey of the United States Department of Agriculture reports it as having been noted in Monroe and

[1] Halstead, B. D. New Jersey Agr. Exp. Sta. Bul. 151. 1901.

Jackson counties of the same state in 1906. Specimens on pods were also collected in Preston Country, Connecticut in this same year by G. P. Clinton. In 1908 it was reported as having caused considerable damage to pole beans and some injury to bush beans at Central Village and Voluntown, Connecticut. In this case the pole beans were killed half way up the poles. The writer has also examined specimens collected by G. P. Clinton at Westville, Connecticut in the years 1902 and 1905. During the summer of 1916 M. F. Barrus found it occurring on beans in the market at Washington, D. C., and the writer has looked over some material which he collected that same year at Chevy Chase, Maryland. Recently also the writer examined some material collected near Managua, Nicaragua, in Central America, by Mrs. G. V. Long, who, not being acquainted with the disease, gathered spotted leaves of the bean at random. Since all the leaves gathered showed this disease, it is probable that it occurs in that locality in considerable abundance.

In spite of this general world distribution, the literature concerning the fungus is meagre. With the exception of Halstead's previously mentioned note of its occurrence and a similar one by Clinton[2] all of the published references to it are brief descriptions of the specimens in exsiccati with the date and place of collection. Furthermore the figures and descriptions are not in accord. Saccardo[3] speaks of the hyphae which contitute the coremium on which the spores are borne as having reflexed tips and his figure[4] represents this character as being very pronounced. Briosi and Cavara[5] have the only other figure that the writer has been able to find with the exception of a copy of Saccardo's in Comes' textbook. This figure does not represent the tips as being at all reflexed and examination of the specimen of Briosi and Cavara shows agreement with their figure in this respect. In all the material that the writer has examined including the greater part of that mentioned previously in this paper no reflexed hyphae have been found.

The Isariopsis leaf-spot is different from the other diseases of the bean in that it is confined more exclusively to the foliage, though in one or two instances it has been reported as occurring on the pods also. On the under side of the leaf are produced numerous small angular spots without a colored border of any sort (fig. 1). It is this angularity of the spots and absence of a colored border that differentiates it so readily from the leaf-blotch of the bean caused by *Cercospora cruenta*. In this latter disease,

[2] Clinton, G. P., Connecticut Agr. Exp. Sta. Rept. **1908**: 308.

[3] Saccardo, P. A. Sylloge Fungorum **4**: 630. 1878.

[4] Saccardo, P. A. Fungi Italici, *tj.* 837. 1878.

[5] Briosi and Cavara, Funghi Parassiti delle Piante Cultivate ed Utili. Fasc. 1. No. 17. Pavia, 1888.

the spots are less angular and are surrounded by a pronounced red-brown border. The middle portions of the spots are also likely to fall out in the older specimens, producing a shot-hole effect. The angularity of the Isariopsis spots is due to the fact that they are bounded and limited by the small veinlets of the leaf. In youth these spots are covered by a gray, moldy coating due to the large number of spores produced but as the spot ages it becomes a pronounced light brown. On close observation it will be seen to be studded with small dots, the coremia, on which the spores are borne in large numbers.

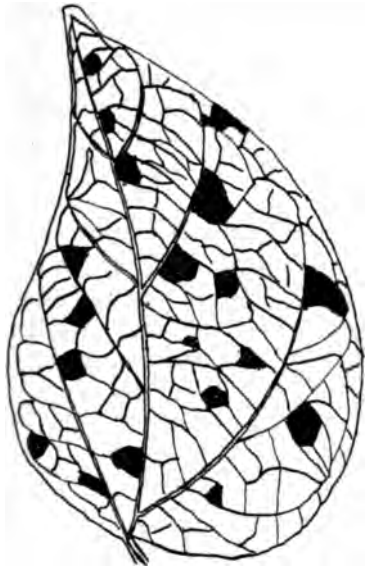


FIG. 1. LEAF OF BEAN SHOWING ANGULAR ISARIOPSIS SPOT

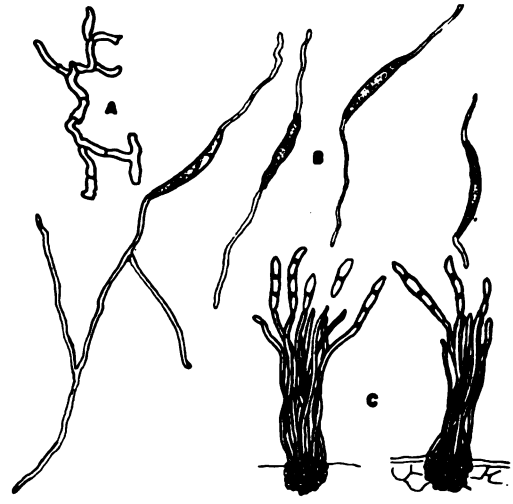


FIG. 2. ISARIOPSIS GRISEOLA
A, mycelium; *B*, germinating spores; *C*, Coremia

The coremia are columnar and are formed of rather dark brownish hyphae closely aggregated, though seemingly not at all united with each other (fig. 2, *C*). The members of the fruiting column tend to separate, especially with age, thus indicating that the structure should perhaps not be regarded as a typical coremium at all. The number of hyphae in a column varies considerably. In some as few as eight hyphae were observed while in others the number often reached thirty or forty. The hyphae are continuous, brownish, becoming paler toward the tip and average about 200 μ in length. The average thickness of the coremium is from 20 to 40 μ.

The conidia are borne on the smooth tips of the hyphae which constitute the coremium (fig. 2, *C*). These tips commonly spread at the top, especially in the older specimens, giving a capitate appearance to the spore cluster. The writer was unable, however, to observe the reflexed tips as described and pictured by Saccardo. The conidia are light gray in color, cylindrical to spindleform, slightly curved, and scarcely, if at all, constricted. They measure 50 to 60 μ in length by 7 to 8 μ in thickness, and are 1- to 3-septate. In a few cases they become 4-septate. The mycelium, though composed of crooked and branching cells, is of practically uniform diameter throughout (fig. 2, *A*). It lives in the leaf tissues and forms dark stromata in the cavities beneath the stomata and from these the coremia arise.

On germination the end cells only, of the 3- or 4-septate spores, send forth mycelial hyphae. These young hyphae are non-septate in the earlier stages and in a short time begin to branch as shown in figure 2, *B*.

No experimental work has yet been done with reference to treatment of the disease, probably because it has not been considered of sufficient economic importance. However, Halstead suggests that, owing to the superficial character of the fungus, the same treatment ordinarily applied to the leaf-blotch or the rust of the bean would probably be effective.

Specimens examined: Rabenhorst, Fungi Eur. 3998; Kunze, Fungi Sel. 595; Briosi and Cavara, Funghi Parassiti, No. 17; Saccardo, P. A., Fungi Ital., Fig. 837; Thümen, Herb. Myc. Sec. 654; Ellis and Everhart, Fungi Columbiani, 2434; Ellis and Everhart, North American Fungi, 2487.

Other specimens collected by: Sheldon, J. F., Morgantown, W. Va., 1186; Martinsburg, W. Va., 1989; Bull. Run, W. Va., 2696; Sink's Grove, W. Va., 2719; Barrus, M. F., Chevy Chase, Md., 9057; Clinton, G. P., Westville, Conn., two packets; Long, Mrs. G. V., Managua, Nicaragua; Stevenson, J. A., Rio Piedras, Porto Rico, 6198; Stevens, F. L., Porto Rican Fungi, Jayuya, 7108; Managuez, 5989; Dos Bocas, 7382; 7952.

CERCOSPORA NICOTIANAE E. & E. ON TOBACCO

Tobacco is third in rank among the exports of Porto Rico, though only a small portion of the area of the island is devoted to its culture. The Cercospora disease may result in great damage to the standing tobacco, and in some instances the crop may be practically ruined. It is most abundant on the lower leaves, appearing as brown, circular spots, from the size of a pin-head to a centimeter or more in diameter, thickly scattered over the entire leaf surface. The older spots are bordered by a dark, raised line, and the centers dry up and become white, often falling

away and leaving irregular holes. The leaf does not decay as a result of the spotting, but turns yellow from the tip downward and ripens prematurely.

Dos Bocas, 7980; Ste. Ana. 7612; Quebradillos, 7270; Caguas, 469; Cailes, 23.

CERCOSPORA HENNINGSII ALESCH. ON CASSAVA

Cassava, *Manihot utillisma*, is cultivated for its thick, fleshy root-stocks, which are densely stored with starch. Although it is not used in Porto Rico nearly as much as it is in some other tropical countries, it is cultivated by the natives to some extent for use as bread and for the starch which it contains. *Cercospora henningsii* causes a small dried-out spot on the leaves, but it is of but little importance, as it probably injures the host but little.

Hormigueso, 233; Santurce, 254.

CERCOSPORA HIBISCI TRACY & EARLE, ON OKRA

Okra is cultivated for its large fruit capsules which are used for food. *Cercospora hibisci* occurs on the lower side of the leaves, not in spots, but as an almost continuous coating. It causes the leaves to turn yellow and fall, weakens the plant and reduces the quantity of pods.

Quebradillas, 5030; Aguadilla, 5229; Cario Raja, 6465.

CERCOSPORA CANESCENS ELL. & MART. ON THE BEAN

Cercospora canescens does some damage to the bean crop, due to small spots on the upper leaf surface, but, as a rule, it does not prove serious.

Guayanilla, 5872.

CERCOSPORA COFFEÆ ZIMM. ON COFFEE

Coffee is by far the most important product of Porto Rico. *Cercospora Coffeæ* causes the appearance on the leaf of spots and the leaves fall, thus reducing the vitality of the plant and preventing the proper maturing of the berries. These spots, as a rule, are round or oval in form, clear brown on the lower side of the leaf, dark brown on the upper side. By uniting, they frequently cause large blotches which dry out and become gray in color at the center. Badly infected leaves appear more or less brown.

Añasco, 3211; Maricao, 4827.

PUCCINIOPSIS CARICÆ EARLE, ON THE PAPAW

The tropical papaw (*Carica papaya*) is attacked by a leaf-spot fungus, *Puccinopsis Caricæ*, which occurs as small, erumpent, black masses on the under side of the leaf, and causes more or less yellowing of the surround-

ing tissue. The attacked leaves die and fall prematurely. The disease is not destructive, but is sometimes reported to be severe on young seedlings. Associated with the causal fungus in all specimens examined by the author was found a very peculiar fungus, *Zygosporium oschioides* Mont., which is represented in figure 3. It occurs as a saprophyte on the Pucciniopsis spots, and is mentioned here only on account of its very interesting peculiarities which are shown in the figure.

Guanica, 348; Vega Baja, 1913; Mona Island, 6334; 6432.

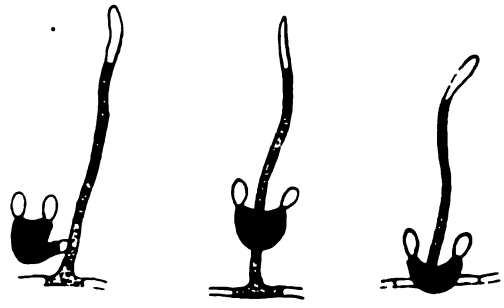


FIG. 3. PUCCINIOPSIS CARICÆ

PHYLLACHORA GRATISSIMA REHM, ON THE AVOCADO

This fungus causes jet-black, erumpent pustules to appear on the upper side of the leaves of the avocado (*Persea americana* Mill.). The spots are from one to two millimeters in diameter, but by coalescing they may become considerably larger. The injury to the plant is probably not very great.

Jayuya, 5974; 6072.

MYCOSPHÆRELLA PERSEÆ MILES (IN ED.) ON THE AVOCADO

This fungus causes very large, irregular spots on the leaves of the avocado. On the upper side of the leaf the spots are covered by a cinereous, papery membrane, and are without a limiting border. On the lower side they are brown, with a narrow border darker brown in color. A single spot may often cover several square centimeters of the leaf surface. The disease is not of any great importance, though it may lessen the vitality of the host somewhat. The description of the fungus may be found in the Proceedings of the Illinois Academy of Science for 1917.

Mariçao, 753; 4809; 4486 (type); Rio Piedras, 2176; 2501; San German, 5797; Dos Bocas, below Utuado, 6601.

CERCOSPORA CARBONACEA MILES (IN ED.) ON THE YAM

This fungus causes very conspicuous, black spots, burned or charred in appearance, on the upper side of the leaves. The spots are usually angular in form, limited by the veins or veinlets, and are from one to one and one-half centimeters in diameter. From the number of collections made in Porto Rico, it would appear to be common there, and it cannot be other than injurious to the plant, since it renders so much of the leaf surface inactive. The description of the fungus may be found in the Proceedings of the Illinois Academy of Science for 1917.

Vega Alta, 4178 (type); Cabo Rajo, 6469; Vega Baja, 4234; Anasco, 3563; St. Ana, 6687.

HELMINTHOSPORIUM MAYAGUEZENSE MILES (IN ED.) ON PASPALUM

Paspalum conjugatum Bregius, a forage grass of some commercial importance in Porto Rico, is attacked by a leaf-spot fungus, *Helminthosporium mayaguezense*. The spots occur both on the blades and culms and are quite conspicuous. They are of uniform light yellow color, surrounded by a narrow, dark brown border, regular in outline, oval, and vary from small to a centimeter in length, being usually about one-half as wide as long. In the somewhat paler centers of the spots the unusually large conidiophores of the causal fungus are plainly evident to the unaided eye as small, dark, hair-like bodies. The spots may be so numerous as to practically cover the leaf and must of necessity be of material injury to the general vigor of the host. The description of the fungus may be found in the Proceedings of the Illinois Academy of Science for 1917.

Mayaguez, 970; 1066; 7142 (type); 8232; 8279; 8941; Dos Bocas, 1093; San German, 5803; Anasco, 4904; Maricao, 8776.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

TWO NEW FOREST TREE RUSTS FROM THE NORTHWEST

H. S. Jackson

CHRYSOMYXA

A large collection of rusts made by Dr. J. R. Weir in various sections of the Northwest, was, through the kindness of Dr. J. C. Arthur, to whom the material was sent in the fall of 1915, referred to the writer for study. Among the material were specimens of a curious rust occurring on the leaves of *Picea Engelmanii* collected in Idaho. Carefully made sections of this form showed that the rust belonged to the genus Chrysomyxa. Examination of undetermined material on the same host in the Arthur herbarium at the Purdue Experiment Station led to the discovery of another specimen of the same rust collected in 1911 in British Columbia by Prof. E. W. D. Holway. Recently Dr. Weir has sent to the writer a third collection made in Oregon in 1916. Since no similar rust has apparently been recorded for America the results of the study of these specimens are considered worthy of record.

The genus Chrysomyxa was established by Unger[1] in 1840 with *C. Abietis* (Wallr.) Ung. as the type species. This is an autoecious leptoform as shown by Reess,[2] telia only, unaccompanied by pycnia being known in the life cycle. This genus has generally been interpreted as including both short and long cycle forms. The Sydows[3] describe sixteen species. Fourteen of these are long cycle forms all of which are known to be, or are assumed to be heteroecious and occur on various members of the Pyrolaceæ, Ericaceæ or Vacciniaceæ with pycnia and aecia so far as determined on Picea. Two short cycled forms *C. Abietis* and *C. Piceæ* Barcl. are included. The former occurs on the leaves of various species of Picea throughout continental Europe; the latter is known only from India on *P. Morinda* and is referred doubtfully to this genus.

Arthur[4] restricted the genus Chrysomyxa to include only the short cycle autoecious forms and established Melampsoropsis (Schröt) for those species having all spore forms. He has recognized[5] eight species of the

[1] Beitrage zur Vergleichenden Pathologie, p. 24. 1840.
[2] Bot Zeit. **23**: 388. 1865; Abh. Naturf. Gesellsch. Halle **11**: 32. 1869.
[3] Monog. Ured. **3**: 502 520. 1915.
[4] Result. Sci. Congr. Bot. Vienne 338. 1906.
[5] N. Am. Flora **7**: 118 121. 1907.

latter genus in America, four of which have been definitely connected through cultures by European or American investigators with their aecial stages. No American representative of the genus Chrysomyxa has been previously recognized.

A careful study of the collections on *Picea Engelmanii*, referred to in the introductory paragraph has led to the conclusion that they represent an undescribed species, a diagnosis of which follows:

Chrysomyxa Weirii sp. nov.

O. Pycnia unknown, probably not formed.

III. Telia foliicolous on yellowish spots, prominent, waxy in consistency, elongate-elliptical, 0.5 to 1.5 mm. long, occasionally confluent, dull orange to orange-brown, ruptured epidermis conspicuous; teliospores catenulate soon separating, oblong or fusiform, 5 to 7 by 16 to 28 μ, truncate or attentuate at either end, abutted or overlapping, sometimes only slightly so at one side; wall colorless, thin 1 μ or less, smooth.

ON PINACEÆ:

Picea Engelmanii Parry. Gold River British Columbia, June 10, 1911, E. W. D. Holway; Priest River, Idaho, May 1915, J. R. Weir *68;* Whitman National Forest, Oregon, July 17, 1913. J. R. Weir *271*, type.

This species differs from *C. Abietis* in the narrower, somewhat smaller spores which do not long remain in chains but soon break apart. No evidence of germination has been seen in any of the collections. The writer takes pleasure in dedicating the species to Dr. Weir who collected the type specimen and whose work has contributed much to a better understanding of Northwestern Uredineæ.

MELAMPSORA

Two species of Melampsora on Populus have been recorded from the Northwest. One of these *M. albertensis* Arth. is apparently confined to *P. tremuloides*. The other, known on various species of Populus, has generally been referred to *M. Medusæ* Thüm. While working over a large collection of rusts from the herbarium of the Montana Agricultural College in the fall of 1915 certain collections were found which did not agree with any species described. A careful study of all the Northwestern and Pacific coast collections has led to the conclusion that all of the material examined on *P. acuminata*, *P. angustifolia*, *P. balsamifera* and *P. trichocarpa* from that region is identical and shows certain distinct morphological characters which enables it to be easily separated from *M. Medusæ* and *M. albertensis*. A diagnosis follows together with a list of the collections in the Arthur herbarium and those from Oregon in the herbarium of the writer.

Melampsora occidentalis sp. nov.

O & I. Pycnia and aecia uncertain, probably on Larix.

II. Uredinia chiefly hypophyllous, scattered, roundish, rather large, 0.5 to 1.5 mm. across, early naked, somewhat pulverulent, orange-yellow, fading to pale yellow, ruptured epidermis conspicuous; urediniospores oblong, ellipsoid or pyriform, 16 to 29 by 32 to 48 μ, slightly flattened laterally; wall colorless or slightly tinted with brown, 2 to 3 μ thick or up to 7 μ on the flattened sides; moderately and prominently verrucose-echinulate, without smooth spots, pores obscure; paraphyses numerous, intermixed with the spores, capitate or clavate, 16 to 24 by 42 to 77 μ, wall colorless, 2.5 to 3 μ thick, uniform, or thickened to 6 μ at apex.

III. Telia chiefly hypophyllous, scattered or more commonly crowded and sometimes confluent in groups surrounding the uredinia, irregularly rounded, small, 0.2 to 0.5 mm. across, slightly elevated at maturity, subepidermal, waxy, at first light cinnamon-brown becoming blackish brown; teliospores prismatic, 10 to 20 by 40 to 64 μ, wall cinnamon-brown, smooth, 1 to 2 μ thick, darker and thicker at apex, 3 to 5 μ; apical pore evident.

ON SALICACEÆ:

Populus acuminata Rydb., II, Stevensville, Montana, Sept. 10, 1908, D. B. Swingle *622;* II, III, Libby, Montana, Oct. 15, 1911, J. R. Weir *40.*

Populus angustifolia Jas., II, Livingston, Montana, Sept. 1, 1913, E. T. & E. Bartholomew (Barth. Fungi Colomb. *4332*, N. A. Ured. *1315*, as *M. Medusæ* Thüm.); II, III, Libby, Montana, Oct. 15, 1911, J. R. Weir; II, Big Horn Mts. Wyoming, Aug. 1898, Williams & Griffiths (Griff. West. Am. Fungi *369* as *M. populina* Lev.); II, III, Willis, Montana, Oct. 1888, J. W. Anderson; II, Seattle (?) Washington, 1906, Bonser *13;* II, Yosemite Valley, California, 1909, P. H. Rolfs.

Populus balsamifera L., III, Sour Dough Canon near Bozeman, Montana, Apr. 1, 1914, H. M. Jennison *88;* II, Sept. 4, 1913, E. Bartholomew (Barth. Fungi Colomb. *4548* as *M. albertensis*); Bremerton, Washington, Sept. 23, 1912, E. Bartholomew (Barth. Fungi Collomb. II, *4434*, III, *4435;* Barth. N. A. Ured. *806* as *M. Medusæ*); II, III, McCarthy Mts., near Willets, Montana, Oct. 1888, F. W. Anderson *383;* II, III, Leonia, Montana, Sept. 14, 1900, J. W. Blankenship *0253, 0254;* II, III, Kalispell, Montana, July 21, 1900, J. W. Blankenship *0255;* II, Caldwell, Idaho, Sept. 28, 1912, E. Bartholomew (Barth. Fungi Colomb. *4034* as *M. Medusæ*); II & III, Madison, Wisconsin, Oct. 14, 1910, E. T. Bartholomew (Barth. Fungi Colomb. *3915* as *M. Medusæ*).

Populus candicans Ait. II, III, Missoula, Montana, Oct. 10, 1898, Williams & Griffiths.

Populus trichocarpa Nutt., II, Corvallis, Benton Co., Oregon, Sept. 1909, H. S. Jackson *1069;* II, III, Oct. 15, 1912, H. S. Jackson *1024*, type; III, March 12, 1916 G. B. Posey; II, III, Trail to Sulphur Springs, Benton County, Oregon, Nov. 2, 1914, H. S. Jackson *3369;* II, Scott's North of Fort Klamath, Klamath Co., Oregon, Sept. 20, 1913, E. P. Meinecke *CrD2;* II, Clatskanie, Columbia Co., Oregon, Oct. 6, 1914, F. D. Bailey *3358;* Oct. 29, 1914, F. D. Bailey *3306;* II, III, Sumpter, Baker Co., Oregon; Aug. 21, 1915, J. R. Weir *265;* II, Medical Springs, Oregon; Aug. 1913, II, III, J. R. Weir *117;* II, Spokane Washington, July 1915, J. R. Weir *42;* II, III, Cour d'Alene, Idaho, Sept. 1915, J. R. Weir *72;* II; Libby, Montana, Oct. 15, 1915, J. R. Weir *40;* II, Pasadena, California, Dec. 29, 1895, A. J. McClatchie; III, Foothills, near Stanford University, Santa Clara, California, April 1, 1902, C. F. Baker *435;* III, Seattle, Washington, Oct. 8, 1892, A. M. Parker *111;* Grizzly Trail, Beaver Valley, British Columbia, July, 1907, E. W. D. Holway; Puyallup, Washington, Aug. 23, 1909, E. Bartholomew (Barth. Fungi Columb. *4143* as *M. Medusæ*); Vernon, British Columbia, 1916, W. H. Brittain; Dillon, Montana, Aug. 3, 1914, J. R. Weir *37;* II, III, Leclede, Idaho, Sept. 1913, J. R. Weir *20.*

This species differs from all other species of Melampsora on Populus in the large size of the urediniospores which are only slightly flattened and are evenly verrucose-echinulate. The teliospores are much longer than those of *M. Medusæ* and are thickened at the apex. The character of the telial sori suggests that this species may be closely allied to *M. albertensis.* The sori are much larger as are also both uredinio- and teliospores.

This species may be the same as that recently cultured by Weir & Hubert[6] who used telial material from *P. trichocarpa* referred to *M. Medusæ* and obtained successful infection on *Larix europea* and *L. occidentalis.* The actual material used for infection and the aecia resulting have not been seen by the writer but telial material sent by Dr. Weir from Montana agrees with the form described above. Aecia from the same locality on *L. occidentalis* agree in general with aecia of *Melampsora Medusæ* and *M. Bigelowii.* The walls of the aeciospores are, however, somewhat thinner, 1 to 2 μ, and considerably thickened on opposite sides to 3 to 5 μ. They measure 17 to 19 by 19 to 26 μ. Additional culture work, and a careful comparison of the resulting aecia with those of *M. Medusæ* would be desirable. In any case, the morphological characters of the uredinial and telial stages are considered sufficient to warrant separation.

PURDUE UNIVERSITY EXPERIMENT STATION
LAFAYETTE, INDIANA

[6] Phytopath. 7: 108. 1917.

RELATION OF TEMPERATURE TO THE GROWTH AND INFECTING POWER OF FUSARIUM LINI

W. H. TISDALE

WITH PLATE XI AND ONE FIGURE IN THE TEXT

Studies on the nature and inheritance of resistance in flax to the wilt disease, caused by *Fusarium Lini* Bolley, were begun with the departments of plant pathology and experimental breeding of the University of Wisconsin in the winter of 1914–15. While growing plants in the greenhouse for these breeding experiments in the winter of 1915–16 the writer noted that marked differences existed in the rate of wilting of susceptible flax plants which were growing at different distances from the heating system. These observations, combined with temperature records, suggested the possibility that soil temperature might be one of the chief factors influencing the rate of infection of these plants. In order to determine this point experiments were arranged so that both the relation of temperature to the growth of the fungus in pure culture, and the relation of soil temperature to the infection of susceptible flax plants might be studied.[1]

RELATION OF TEMPERATURE TO THE GROWTH OF THE FUNGUS IN CULTURE

After ascertaining by careful measurement that actual correlation existed between soil temperatures and the rate of wilting of the flax plants, a study was undertaken of the relation of the fungus to various temperatures while growing in pure culture on artificial media. An investigation of this kind was considered of fundamental importance in order to work out and understand thoroughly the relation of soil temperature to infection. By using an incomplete series of temperatures ranging from 0 to 37°C. it was found that the organism failed to grow below 10°C. and at or above 37°C. This preliminary experiment showed approximately the temperature limits of the fungus and gave some indications as to what temperatures were best suited for its growth. Another experiment was arranged, using a more complete series of temperatures, in which plates

[1] The writer wishes to express his hearty appreciation to Professor H. L. Bolley of the North Dakota Agricultural Experiment Station for supplying flax seed and flax sick soil for the work. He is also indebted to Professor L. R. Jones of the University of Wisconsin for invaluable suggestions and kindly criticisms of the work as it progressed.

poured with a 1.8 per cent potato agar and inoculated in the center with bits of mycelium were incubated in duplicates at different temperatures for six days. After incubation the colony diameters were measured (table 1) and the plates arranged in a temperature series and photographed (plate XI). The plate numbers in table 1 correspond to the numbers in the photograph.

TABLE I

The effect of temperature on the growth of Fusarium Lini. Cultures six days old

PLATE NO.	TEMPERATURE	DIAMETER OF COLONY
	°C.	mm.
1	8.5–10	0
2	11	7
3	12–13	20
4	14	25
5	15	29
6	16	34
7	17	38
8	19	43
9	22	68
10	24–25	70
11	25–26	72
12	26–28	78
13	29–30	75
14	34	17
15	37	0

It appears from table 1 that the minimum temperature for growth of the fungus lies between 10° and 11°C., the optimum at about 26° to 28°C., and the maximum between 34° and 37°C. Another set of experiments showed that the fungus is able to grow slightly at a temperature ranging from 35° to 36°C. Judging from the vigor of the fungus at the various temperatures, it would be expected that flax plants would wilt more readily with soil temperatures between 20° and 30°C. This was actually found to be the case with susceptible plants growing in the greenhouse.

RELATION OF SOIL TEMPERATURE TO INFECTION[2]

While growing flax plants in the greenhouse for breeding experiments in the winter of 1915–16, as previously stated, a series of flats was placed near a system of heating pipes, while others were placed at a greater distance from the pipes where the temperature was lower. A more rapid

[2] Tisdale, W. H. Relation of soil temperature to the infection of flax by Fusarium Lini Boll. (Abst.) Phytopath 6: 412. 1916.

wilting of susceptible plants was noticed in flats near the heating system. Soil temperatures were taken and it was found that the temperature in flats near the heating pipes ran at 18° to 20°C., while the temperature in flats farther from these pipes, where there was much less infection, ran at 14° to 17°C. This seemed to indicate that the critical temperature for the infection of flax by *Fusarium Lini* is somewhat below 17°C., which was of the more interest, in view of the fact that Gilman found that the lowest temperature for the infection of cabbage by *Fusarium conglutinans* Wr. is about 17°C.

Following these observations an attempt was made by controlling the soil temperature to determine more accurately the lowest temperature for infection of flax by *Fusarium Lini*. The first attempt was made by placing pots of infected soil containing germinating flax seeds in the cold-storage cellar near a small window where they could get light. Other pots planted at the same time were kept in the greenhouse as controls. The temperature in the cellar often ran below 10°C. during the nights. This temperature was too low for the plants to remain in a vigorous condition. The plants in the control pots began wilting in ten days and nineteen of the twenty-two plants were wilted at the end of twenty-two days, whereas none of the plants at the lower temperature in the cellar had wilted. The experiment was then terminated by a sudden drop in temperature which froze the plants in the cellar.

A second attempt was made at controlling the soil temperature by arranging a cool, circulating water jacket (fig. 1). A six-inch earthenware jar was filled about three-fourths full of infected soil and susceptible flax seeds planted. This jar was then supported in a larger jar, through which cold water flowed continuously, as shown in the figure. Seeds were planted in a similar jar and placed nearby at greenhouse temperature as a control. At the time these experiments were begun the temperature of the water was about 9°C. By careful regulation of the flow by means of the faucet valve it was possible to hold a fairly constant temperature between this point and the greenhouse temperature. A temperature ranging between 12° and 15°C. was maintained for eight days and the plants remained free from infection. The temperature was then raised to 16° to 17°C. for two days and lowered again to 12° to 15°C. Four of the twenty-three plants in the jar showed signs of the wilt within two days even at these low temperatures. Infection evidently occurred during the brief period at the higher temperature. The small earthenware jar was found to be unsatisfactory due to the fact that the material allowed too much condensation or seepage which kept the soil almost saturated. The experiment was then repeated with a glass battery jar substituted for the earthenware jar. The glass jar proved more satisfactory. In the second

experiment it was found that at temperatures below 15°C. no wilting of flax plants occurred, while plants in the control pot at temperatures ranging from 19° to 21°C. wilted rapidly. During the earlier part of this trial when the temperature was held at 13° to 14°C. the flax plants grew fairly well and no indications whatever of wilt were evident (fig. 1, A). After one month, when the temperature had risen to 15°C. only one plant of the twenty-one in the cold jar showed any sign of infection, while all of the thirty-three plants in the control jar were killed by wilt. The temperature in the cold jar could not be lowered as the water was warming up with the coming spring.

FIG. 1. RELATION OF TEMPERATURE TO THE INFECTION OF FLAX BY F. LINI

A, Susceptible flax plants growing at 13° to 15°C. in infected soil in a glass jar surrounded by running water. The smaller jar is supported by a glass tumbler inverted on the bottom of the larger jar. *B*, Susceptible plants growing in infected soil at greenhouse temperatures (19°–21°C.). Plants in this jar are practically all dead with wilt.

It seems from these experiments that the critical temperature for infection of flax by *Fusarium Lini* is between 14° and 16°C. This conclusion accords well with the previous evidence secured by culturing the fungus on agar at graduated temperatures. As stated in table 1 the minimum temperature was found to be 10° to 11°C. and reference to plate I will show that but little growth occurred below 15°C. We should expect the critical temperature for infection to be somewhat higher than the minimum tem-

perature at which the parasite will grow, especially since the host plant thrives well at the lower temperatures. In this case it seems clear that the host plant which is susceptible at the higher temperatures is able to resist or overcome whatever weak attempts at invasion the fungus can make at the lower temperatures.

The evidence here presented supplementing that secured by Gilman[1] should at least warrant further careful attention to the relation of temperatures to infection with the soil parasites, and especially the species of Fusarium.

CONCLUSIONS

1. *Fusarium Lini* grows on culture media at temperatures between 10° and 37°C., with its optimum temperature at 26° to 28°C.

2. Flax thrives well with soil temperatures as low as 13°C.

3. The critical temperature for the infection of flax by *Fusarium Lini* is about 15°C.

4. There is a close correlation between the temperatures at which *Fusarium Lini* grows best in pure culture and those at which flax wilt is most destructive.

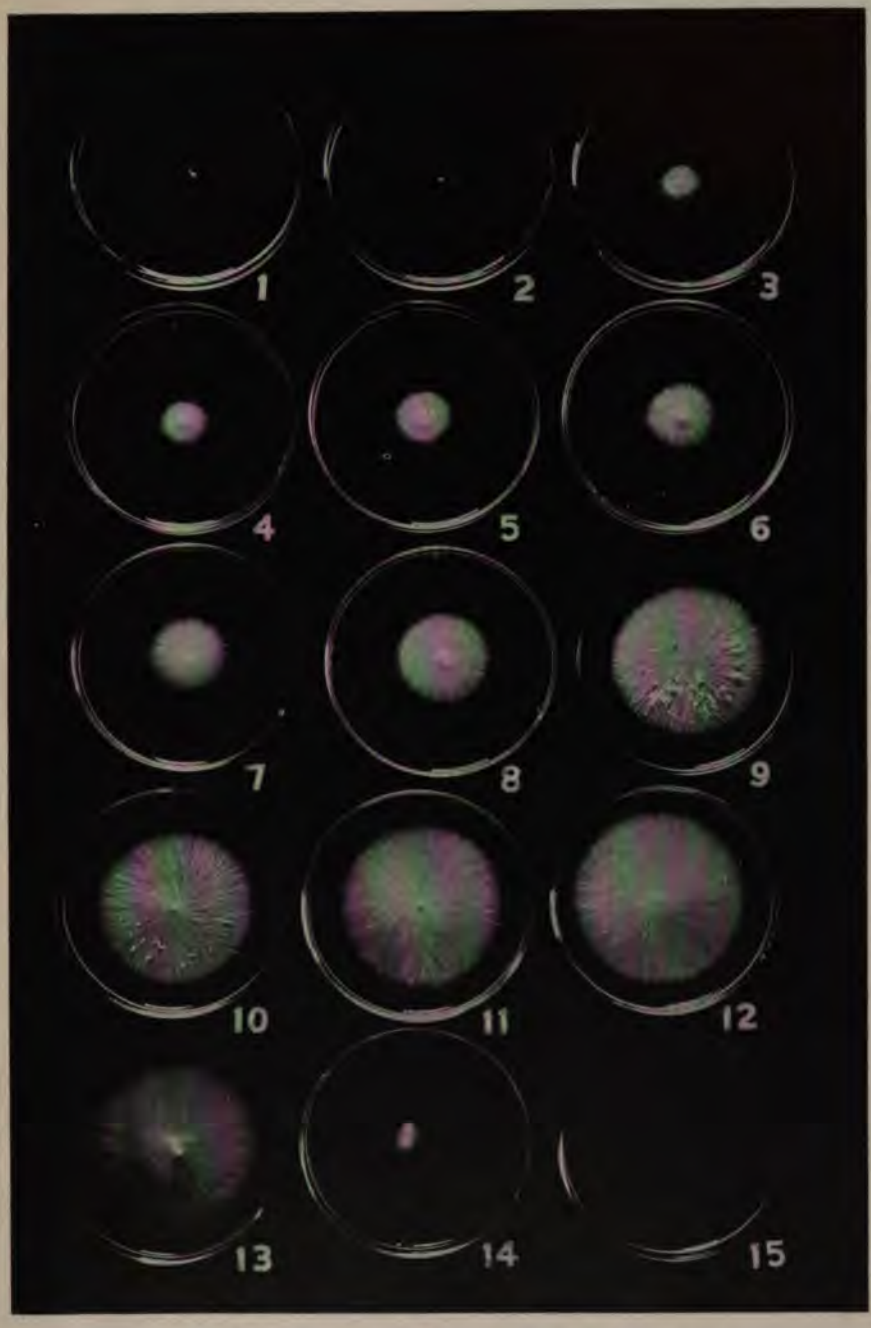
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

[1] Gilman, J. C. The relation of temperature to the infection of cabbage by Fusarium conglutinans Wollenw. Phytopath. 4: 404. 1914.

—— Cabbage yellows and the relation of temperature to its occurrence. Ann. Missouri Bot. Gard. 3: 25-84. 1916.

PLATE XI. RELATION OF TEMPERATURE TO THE GROWTH OF FUSARIUM LINI

These culture plates (1.8 per cent potato agar) were inoculated with much care as to uniformity and then incubated for six days at different temperatures, as follows: 1, 8.5°; 2, 11°; 3, 12-13°; 4, 14°; 5, 15°; 6, 16°; 7, 17°; 8, 19°; 9, 22°; 10, 24-25°; 11, 25-26°; 12, 26-28°; 13, 29-30°; 14, 34°; 15, 37°.



TISDALE: RELATION OF TEMPERATURE TO INFECTION

A SIMPLE AND EFFECTIVE METHOD OF PROTECTING CITRUS FRUITS AGAINST STEM-END ROT

JOHN M. ROGERS AND F. S. EARLE

During the summer and fall of 1914, while studying the various rot organisms of citrus fruits at San Pedro, Isle of Pines, some very interesting facts were noted.

The percentage of rot in transit during the previous year was abnormally high and in the year of 1914 studies were made of the various conditions and organisms to ascertain the causes. It was decided at first that improper handling of the fruit had had much to do with the high percentage of decay during the season of 1913. In 1914, however, the fruit was handled in the most careful manner, having been picked under direct supervision of competent foremen, by laborers wearing cotton gloves. The fruit was placed in standard field crates and hauled to the packing house on wagons with bolster springs. The fruit was graded by expert graders and every doubtful fruit was culled out. Despite this careful handling a large amount of decay occurred in transit, but the rot was confined almost entirely to the variety of grapefruit known on the Isle of Pines as the Pinero or Native, a variety very similar to the Florida Triumph.

Counts were made of the rotten fruit from the culls and it was found that over eighty-three per cent of the rot was caused by a species of Diplodia. In many cases the organism had gained entrance through some injury, but in the majority of the fruit the rot started from the stem end with no apparent injury.

Inoculation experiments were made with the spores and hyphae of the Diplodia and it was found that perfectly sound fruit in all conditions of maturity could be rotted down through the stem end if moisture conditions were favorable. In this connection it was noted that the fruit that had been clipped showed more rot in the inoculation experiments than did the fruit that had been pulled, thereby removing the calyx. This was accounted for by the fact that the small cavities beneath the calyx under humid conditions, do not dry out quickly and make an excellent infection court. The small portion of stem left when the fruit was clipped, frequently became infected and through this source the infection entered the fruit. When the fruit was pulled the stem cavity had an opportunity

to dry quickly and there was less chance of spores lodging and germinating than where the calyx had not been removed.

With these facts in mind some method was sought of sealing the stem ends to prevent the entrance of the organism. Paraffin was first tried.

Fruit was gathered from trees in poor condition and showing some

TABLE 1

Showing numbers of fruits rotted on various dates in each of the lots

BEGINNING SEPTEMBER 20	LOT 1	LOT 2	LOT 3	LOT 4	LOT 5	LOT 6	LOT 7	LOT 8
September 27	1	0	0	1	0	0	0	0
October 4	3	0	2	0	2	0	0	0
October 10	7	2	5	1	1	1	0	0
October 14	2	4	0	0	2	0	0	0
October 16	1	0	1	0	0	1	0	1
October 20	0	0	0	0	0	0	0	0
November 1	2	2	3	1	1	0	0	1
November 10	1	0	4	2	0	1	2	0
November 19	0	0	1	1	2	0	0	0
Total	17	4	20	6	8	3	2	2

Diplodia die-back. Eight lots were made of twenty fruit each and treated in the following manner:

Lot 1. Fruit clipped and rind paraffined.
Lot 2. Fruit pulled and rind paraffined.
Lot 3. Fruit clipped. (Check.)
Lot 4. Fruit pulled. (Check.)
Lot 5. Fruit clipped, rind and stem end paraffined.
Lot 6. Fruit pulled, rind and stem end paraffined.
Lot 7. Fruit clipped, stem end paraffined and rind not paraffined.
Lot 8. Fruit pulled, stem end paraffined and rind not paraffined.

The number of rotted fruits was recorded and is shown in table 1

Many decayed fruits appeared in the lots that were clipped and the results (table 1) substantiated previous knowledge of the fact that the clipped fruit did not hold up as well as pulled fruit. The difference between the pulled and the clipped lots is so great that it seems as though some other factor must have been partially responsible for the very high per cent of decay.

The treatment of the stem ends gave very promising results and the percentage of rot in the treated lots was greatly reduced. The paraffining of the rind gave no particular protection against rot, but this treatment did bring to light another point of much interest and economic value.

The fruit in the lots that had the rind paraffined did not shrivel and the rind did not dry out as did the other lots not so treated.

There have been two serious troubles in connection with the production of lemons on the Isle of Pines. These have been Diplodia stem-end rot, and the quick shriveling and drying of the fruits. The lemons are so thin-skinned that the rind dries out and becomes hard in a short period of time. By giving the fruits a good coating of paraffin it has been possible to keep them in excellent condition for from two to three months with practically no shriveling.

On October 17, 1915, lemon fruits were pulled and were divided into two lots of fifty fruits each. The stem ends of one lot were paraffined, the others were not. On January 24, 1916, there were a total of nine rotted

TABLE 2

Showing number of rotten fruit on various dates in each of the lots

BEGINNING NOVEMBER 19	LOT 1	LOT 2	LOT 3	LOT 4	LOT 5	LOT 6
December 10	0	4	20	16	8	18
December 30	0	8	8	6	6	8
January 26	1	3	18	4	4	10
Total	1	15	46	26	18	36

fruits in the paraffined lot and twenty-one in the untreated lot. These data and those in table 1 show clearly that the treatment of the stem ends is beneficial in the prevention of rot. Paraffin gave very promising results, but was not altogether satisfactory because it did not stick well and it was therefore difficult to seal the stem ends perfectly.

The next material to be tried was shellac. It was diluted to a thin consistency with alcohol. This material gave such satisfactory results that it was not necessary to seek further. It is easily and quickly applied, sticks well and seals the stem cavity perfectly.

The first experiment to be made with the shellac treatment consisted of six lots of fifty-six fruits each. The lots were treated as follows:

Lot 1. Fruit pulled and stem end shellacked.
Lot 2. Fruit clipped and stem end shellacked.
Lot 3. Fruit clipped and a cut made in the rind with a knife.
Lot 4. Fruit clipped, rind cut with a knife and shellacked. Stem end shellacked.
Lot 5. Fruit pulled. (Check.)
Lot 6. Fruit clipped. (Check.)

The number of rotted fruits was recorded on various dates and is shown in table 2.

The results shown in table 2 are very striking, particularly between lots 1 and 2, and 5 and 6. The results of lots 3 and 4 show that fresh injuries to the skin of the fruit can be protected to some degree by covering the injury with a thin coating of shellac. Better results would have

TABLE 3

Showing number of rotten fruit on various dates in each of the lots

BEGINNING DECEMBER 12	LOT 1	LOT 2	LOT 3	LOT 4	LOT 5	LOT 6
Native grapefruit						
December 20	0	1	1	2	1	2
January 15	0	1	3	5	1	1
February 4	1	2	1	0	0	3
Total	1	4	5	7	2	6
Walter's grapefruit						
December 20	0	0	2	1	0	0
January 15	0	2	1	2	1	2
February 4	1	2	1	2	0	1
Total	1	4	4	5	1	3
Valencia oranges						
December 24	0	0	0	1	0	0
January 15	0	1	2	2	0	0
February 4	0	1	1	3	0	1
Total	0	2	3	6	0	1
Villa Franca lemon						
December 24	0	0	1	1	0	0
January 15	0	1	0	1	0	0
February 4	0	0	1	2	0	3
Total	0	1	2	4	0	3
Persian lime						
December 24	0	6	0	0	0	2
January 15	0	0	2	6	5	4
February 4	1	2	0	2	0	1
Total	1	8	2	8	5	7

undoubtedly been secured in these lots had the fruit been pulled instead of being clipped.

The next experiment was made in order to determine the effectiveness of the treatment on the various kinds of citrus fruits. Twenty fruit were used in each lot and the lots treated as follows:

Lot 1. Fruit pulled, stem ends shellacked, dipped in a suspension of spores of Diplodia.
Lot 2. Fruit clipped, stem ends shellacked, dipped in a suspension of spores of Diplodia.
Lot 3. Fruit pulled and dipped in suspension of Diplodia spores.
Lot 4. Fruit clipped and dipped in suspension of Diplodia spores.
Lot 5. Fruit pulled. (Check.)
Lot 6. Fruit clipped. (Check.)

The results of the experiment are shown in table 3.

The results of the experiment show that the fruits of some varieties and species of Citrus are more subject to the Diplodia rot than others, but that the treatment is just as effective in all cases. The Native grapefruit and the Persian lime showed the highest percentage of rot in this experiment, but the lemon has been found to be very subject to the Diplodia rot, particularly during the curing process.

The fruit when dipped in an aqueous suspension of Diplodia spores showed more rot than the check lot. The shellac gave protection even when the fruit was dipped in the spore suspension.

In the next experiment two boxes of Native grapefruit were sent to Dr. C. L. Shear, Washington, D. C. The fruit was packed and in transit fully two weeks before being opened. It was fully ripe when picked, in fact, some of it was nearly ready to drop from the tree and the trees were in more or less weakened condition from Diplodia die-back disease. This was the most severe test that the shellacking treatment could be subjected to, as the fruit was too ripe to warrant shipping.

One box of fruits was washed by the overhead sprinkler and revolving brush system, with clean running water, no soaking tank being used. Half of the fruit in this box was shellacked and the other half was not. The second box, after being run through the sizers and polishers, was packed dry. Half of the fruit in this box was shellacked and half was not. The fruit in all four lots was pulled. The results, as tabulated by Doctor Shear, appear in table 4.

The results shown in this table were most satisfactory from several standpoints. The shellacking treatment reduced the amount of rot over 25 per cent in both the dry and the washed lots. Washing fruit, even with clear running water, greatly increases the amount of rot from the stem-end organism on many kinds of citrus fruits.

TABLE 4

Report on grapefruit received from Mr. Rogers, February 7, 1917

DATE	DRY		WASHED	
	Stem end shellacked (48)	End not shellacked (48)	Stem end shellacked (36)	End not shellacked (36)
February 9, 1917.	1 rotten. Started in wound	1 rotten. Wholly rotten covered with Penicillium*	1 rotten. Wholly rotten. Seems to have started from wound	17 rotten. 10 all rotten. Penicillium present; 7 partly rotten. Decay apparently at stem end
February 19, 1917	0	4 rotten. 3 apparently started from stem end. 1 from wound; all show Penicillium	3 rotten. 2 rotten, began at stem end. 1 began at bottom end	7 rotten. 5 involve stem end; 2 entirely rotten show Penicillium
March 1, 1917....	1 rotten	3 rotten	4 rotten	13 rotten. 6 all rotten; 5 stem-end-rot; 2 doubtful
March 10, 1917...	2 rotten	7 rotten. 5 typical Diplodia rot	3 rotten	2 rotten
Total	4	15	11	39

* The presence of the Penicillium mentioned does not necessarily indicate that this was the primary cause of rot. It probably followed and obscured the Diplodia.

SUMMARY

Citrus fruits subject to stem-end rot may be protected to a great degree by shellacking the stem end.

If the shellacking treatment is to be most effective, it is necessary that the fruit be pulled and not clipped. In this connection it should be stated that the pulling of grapefruit without tearing a portion of the rind is a difficult undertaking, though it can be accomplished. Oranges, lemons and limes may be pulled more easily than clipped and with no injury to the rind.

Washing the fruit increases the amount of decay to a very great extent. The use of a soaking tank, where the stem-end rot organism is present, is the greatest possible folly.

A stemless lemon, having been pulled and shellacked, will hold up as long as a lemon with the stem intact.

Fruit may be practically guaranteed against rot if given this treatment and handled properly.

Avocados, watermelons and other fruits could possibly be protected against stem-end rot if given this treatment.

Some citrus fruits are more subject to the attacks of Diplodia rot than others. In this locality the Pinero or Native grapefruit, the Persian lime and the Villa Franca lemon suffer most from this rot.

A thin coat of paraffin prevents shriveling and drying, and keeps the fruit in a marketable condition from a month to six weeks longer than fruit not so treated.

SAN PEDRO, CITRUS PATHOLOGICAL LABORATORY

ARTHROPODS AND GASTEROPODS AS CARRIERS OF CRONARTIUM RIBICOLA IN GREENHOUSES

G. Flippo Gravatt and Rush P. Marshall

The literature on dissemination of fungi in general by insects has been recently summarized by Studhalter and Ruggles.[1] Numerous writers have called attention to insects as possible carriers of rust spores, or have shown that rust spores actually were so carried. Wolf[2] notes that vast numbers of urediniospores of the morning glory rust, *Puccinia cassipes* B. & C., were present in the fecal deposits of a katydid. So far as can be determined no previous information has been noted on the dissemination of rust fungi by Gasteropods.

Studies with insects, sow bugs, and snails allowed to feed on various species of the genus Ribes grown for inoculation experiments with *Cronartium ribicola* Fisch. in the pathological greenhouses at Washington, D. C. during the past winter, have shown that these animals have a decided preference for the infected leaves. Observation indicated that the greater part of the feeding was done by a greenhouse weevil, but on leaves growing close to the ground, snails, slugs, and sow bugs were perhaps more active. A small slug was observed eating the telial columns of *Cronartium ribicola* from the under side of a black currant. Apparently, the uredinial pustules and the leaf tissue were untouched. Judging by the thorough manner in which the telial columns had been eaten off the leaves of this bush, the slug had been at work for some days. In the greenhouse, on a white pine (*Pinus strobus*), infected by *Cronartium ribicola*, a sow bug has been observed eating out pycnial pustules and the surrounding tissue of the blister-rust swellings.

SPORES ON THE BODIES OF ANIMALS

Ants, weevils, and one slug were collected directly from infected plants in the greenhouse. In most cases, however, the suspects were confined on Ribes bushes under bell glasses or in dishes with infected leaves. Each individual was removed from the leaves with flamed tweezers to a small

[1] Studhalter, R. A., and Ruggles, A. C. Insects as carriers of the chestnut blight fungus. Pennsylvania Dept. Forestry Bul. 12: 33. 4 *pl.* 1915.

[2] Wolf, F. A. Further studies on peanut leafspot. Jour. Agr. Research 5: 891-902. 1916.

TABLE 1

Examination for spores of Cronartium ribicola on the bodies of animals

DATE 1917	ANIMAL*	PLACE COLLECTED OR CONFINED	TOTAL NUMBER UREDINIO-SPORES IN WASH WATER	NUMBER SPORIDIA FROM TELIAL COLUMNS IN WASH WATER
January 11.....	1 weevil. *Pantomorus fulleri*	On infected Ribes plant under bell jar	21	2
January 11.....	1 weevil, *Pantomorus fulleri*	On infected Ribes plant under bell jar	85	10
January 24.....	1 weevil, *Pantomorus fulleri*	Confined in dish with infected leaves	425	0
January 31.....	1 slug, *Agriolimax agrestis*	Confined in dish with infected leaves	0	0
January 27.....	1 slug, *Agriolimax agrestis*	On infected plant in greenhouse	10	110
January 10.....	5 red ants, *Pheidole anastassii*	On infected plant in greenhouse	25	0
January 17.....	12 red ants, *Pheidole anastassii*	On infected plant in greenhouse	200	0
January 25.....	1 red ant, *Pheidole anastassii*	Confined in dish with infected leaves	0	8
January 25.....	1 red ant, *Pheidole anastassii*	As above	5	135
January 25.....	1 red ant, *Pheidole anastassii*	As above	0	220
January 25.....	1 red ant, *Pheidole anastassii*	As above	0	0
January 25.....	1 red ant, *Pheidole anastassii*	As above	0	20
January 25.....	1 sow bug, *Armadillidium vulgare*	As above	3	85
January 25.....	1 sow bug, *Armadillidium vulgare*	As above	0	450
February 2.....	1 sow bug, *Armadillidium vulgare*	As above	27	0
February 25.....	1 small spider	As above	0	30

* Identifications through the kindness of Paul Bartsch, W. Dwight Pierce and J. R. Horton.

TABLE 2

Tests of adherence of spores to bodies of animals

ANIMAL	TREATMENT AFTER BEING DIPPED	NUMBER DAYS BETWEEN TREATMENT AND EXAMINATION	UREDINOSPORES ON BODY	SPORIDIA ON BODY	PROMYCELIA OR TELIAL COLUMNS
Sow bug, *Armadillidium vulgare*	In petri dish on filter paper	1	2	35	4 promycelia, 1 telial column
Sow bug, *Armadillidium vulgare*	In petri dish on filter paper Transferred to clean quarters on second day	7	0	13*	
Sow bug, *Armadillidium vulgare*	In beaker with soil bottom	2	5	17	
Sow bug, *Armadillidium vulgare*	In beaker with soil bottom Transferred to clean soil on second day	7	0	2*	
Cockroach, *Blatta orientalis*	In petri dish with filter paper	1	4	20	
Cockroach, *Blatta orientalis*	In petri dish with filter paper Transferred to clean quarters on second day	2	0	0	
Cockroach, *Blatta orientalis*	Transferred to clean quarters on second day	4	0	0	
Red ant, *Pheidole anastasii*	On soil	1	4	10*	
Red ant, *Pheidole anastasii*	On soil	2	0	6	

* Several sporidia in these counts were partially germinated. A number of the sporidia were in this condition when insects were treated.

vial containing a measured quantity of distilled water and thoroughly washed to free any adhering spores.

At least one-tenth of the water secured from the first washing was examined under the microscope, and the spores counted. This result was compared with the examination of the wash water as a whole. In a few cases modifications were made of the results obtained by counting one-tenth of the total amount. The figures given in the tables are only approximately accurate, due to the method of counting and the fact that all of the spores were not removed from the bodies by the first washing.

TABLE 3

Examinations for spores of Cronartium ribicola in excreta

DATE 1917	ANIMAL	LOCATION OF ANIMAL BEFORE EXAMINATION	UREDINOSPORES	SPORIDIA	PARTS OF TELIAL COLUMNS
January 11.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	120	0	
January 11.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	10	0	
January 12.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	1,870	210	
January 12.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	780	0	
January 12.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	400	60	
January 12.....	Weevil, *Pantomorus fulleri*	On Ribes bush under bell jar	210	0	
January 27.....	Slug, *Agriolimax agrestis*	On Ribes bush in greenhouse	7	17	16
January 27.....	Slug, *Agriolimax agrestis*	On Ribes bush in greenhouse	7	28	28
January 27.....	Slug, *Agriolimax agrestis*	On Ribes bush in greenhouse	20	180	128
January 27.....	Slug, *Agriolimax agrestis*	On Ribes bush in greenhouse	8	65	96
February 15.....	Slug, *Agriolimax agrestis*	Confined on infected leaves	1,050	0	
February 15.....	Slug, *Agriolimax agrestis*	Confined on infected leaves	960	0	
February 15.....	Slug, *Agriolimax agrestis*	Confined on infected leaves	120	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	2	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	9	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	50	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	0	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	60	0	
February 3.....	Snail, *Subulina octona*	Confined on infected leaves	30	0	
February 2.....	Sow bug, *Armadillidium vulgare*	Confined on infected leaves	750	0	
February 2.....	Sow bug, *Armadillidium vulgare*	Confined on infected leaves	300	0	

Several ants, sow bugs, and cockroaches were thoroughly shaken in a water suspension of urediniospores and sporidia and after being dried were confined in various dishes to determine how long, under certain conditions, the spores would remain on their bodies. The methods used in making examination for spores were the same as those previously given.

SPORES IN EXCRETA

In determining if the entire spores pass through the alimentary tract, the animals tested were either taken while feeding on diseased plants in the greenhouse, or were confined with fungus-bearing material. After being thoroughly washed in several changes of distilled water to remove any adhering spores, they were confined in petri dishes with blotting paper in the bottom to collect excreta. Deposits were removed singly and each macerated in a small quantity, usually ten drops, of distilled water. At least one-tenth of this was carefully examined under the microscope for spores. When the count was not high the entire quantity was examined, rather than a part. Due to the difficulty in counting, and the fact that in many cases only a portion of the material was used, counts were only approximately accurate.

VIABILITY OF EXCRETED SPORES

Germination tests were made of the urediniospores recovered from each excretal deposit, using distilled water at about 13°C. in watch glasses or hanging-drop cells. Of the urediniospores, only those passed by the slug in the first and second pellets of February 5, 1917 gave germinations. The results showed less than one per cent germination, which is considerably lower than the average germination secured with spores of the same age when taken directly from infected leaves. Alternaria spores which passed through the sow bug on February 3, 1917 showed abundant germination. Inoculations made from the first and second pellets of the weevil, collected January 12, 1917 gave positive results when inoculated on one unidentified western species of *Ribes*. Another western species and two plants of *Ribes tenuifolium*, together with the check, remained healthy.

The four excretal deposits obtained on January 27, 1917 from the slug were composed by volume, respectively, of 25, 33, and 95 per cent of parts of telial columns. Germination tests of these portions of telial columns gave an average germination of only a little over three per cent, considering the germination of telospores of a column as the unit. Nearly all of these columns had been cut into several pieces by the slug. Tests made with fresh, whole, telial columns and with fresh, broken, columns show only slight differences in the percentages obtained. As the average ger-

mination of telial columns tested a week after they were removed from the leaf is above 50 per cent, it may be concluded that alimentation greatly lessens the viability of the teliospores.

DISCUSSION

Cronartium ribicola produces abundant urediniospores and teliospores under greenhouse conditions but spreads very slightly during the winter, the new infections being confined to infected plants having leaves within an inch or less of the ground, where moisture remains on leaves for some time after watering. The importance of Arthropods and Gasteropods in causing new infections of the fungus in the greenhouse is probably very slight.

In the field, wind must play the major part in the dissemination of *Cronartium ribicola*, as various tests in and around a diseased Ribes planting show that urediniospores are in the air in decreasing frequency up to fifty feet from the nearest bush and, of course, are carried by strong winds for indefinite distances. However, it seems fairly certain from these results obtained with *C. ribicola*, and those of other investigators of insects and birds with various fungi, that animals are important agents in the dissemination and spread of this fungus.

SUMMARY

1. The small animals tested were bearers of numerous urediniospores and sporidia of *Cronartium ribicola*.

2. Urediniospores and sporidia will adhere to bodies of the animals under certain conditions for at least a week.

3. Small animals feed on the different spore-stages of the blister-rust fungus. Their excreta contain abundant urediniospores and in some cases sporidia and pieces of telial columns.

4. Alimentation lessens the viability of both the uredinio- and teliospores.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
WASHINGON, D. C.

PRELIMINARY REPORT ON THE VERTICAL DISTRIBUTION OF FUSARIUM IN SOIL[1]

MINNIE W. TAYLOR

For the past three years considerable difficulty has been experienced with the damping-off of seedlings of *Pinus resinosa* Ait. and *P. ponderosa* Dougl. in the seed-beds of the pine tree nursery at the Metcalf Botanical Garden of Brown University, Providence, Rhode Island.

The Botanical Garden was originally farm land. It has been uncultivated for some time although the hay is cut each year. The top soil is moderately light colored, sandy loam about 14 inches deep. Below this there is a bed of light yellow, coarse sand from 4 to 10 feet deep underlaid by hard pan. The land in the vicinity of the seed-beds slopes very gently to the northeast.

So far as the writer can learn *Fusarium* has never been definitely reported much more than 7 inches below the surface of the ground. The lowest depth at which Jensen[2] collected soil samples appears to have been 8 inches, but there is no specific mention at what depths the various fungi occurred. Waksman[3] in speaking of the depths at which fungi, including *Fusarium*, were found states that "most of the other organisms were isolated from the upper 8 inches of soil," but as he does not say just what these were, it is impossible to tell whether *Fusarium* was in this group. Beckwith[4] collected all of his samples of soil 2 inches below the surface. Manns[5] mentions experiments with "sick soil" in the greenhouse, but does not state that the soil was taken at any particular depth. He believes that the rate of penetration of *Fusarium* through the soil depends upon the species of *Fusarium*, that of potato blight being

[1] The investigation was conducted in the laboratory of the Botanical Department of Brown University, during the year 1915 1916, as a partial fulfillment for the degree of Master of Arts. The writer wishes to express her appreciation to Dr. Harlan H. York for his kind direction and assistance during the progress of the work

[2] Jensen, C. N. Fungous flora of the soil. Cornell Agr. Exp. Sta. Bul. 315 1912.

[3] Waksman, Selma A. Do fungi live and produce mycelium in the soil? Science, n s 44: 320 322. 1916

[4] Beckwith, T. D. Root and culm infection of wheat by soil fungi in North Dakota. Phytopath 1: 169 176. 1911.

[5] Manns, T F. Fusarium blight and dry-rot of the potato. Ohio Agr. Exp. Sta Bul 229. 1911

slower than that of flax wilt or cabbage wilt. Werkenthin[6] took samples from 1 to 7 inches below the surface and says that "in deeper regions below 4 inches, no viable fungous spores seem to be present." He adds that "of special interest in the study of soil fungi is the fact that the virgin soil contained fungi which are known to be parasitic to cultivated plants, e.g., *Fusarium Solani* (Mart.) Sacc., *F. oxysporum* Schlecht. and *F. radicicola* Wollenweber." In summarizing he says that "pathogenic fungi, especially species of *Fusarium*, live in the soil as saprophytes throughout the winter."

In a large number of cultures, on nutrient agar, which have been made from pine seedlings that had damped off, *Fusarium* sp.[7] was the only fungus found that is known to cause damping-off. Moreover, it was observed that in the seed-beds made from sand taken 18 to 24 inches below the surface of the ground, damping-off occurred just as abundantly apparently as in the beds made from surface soil.

The beds in all instances were made in the usual way. Because of this fact, the present problem was suggested, namely to determine to what depth *Fusarium* occurs in the soil of the nursery and also whether it is present at different places in the grassland surrounding the nursery. The following is merely a preliminary report, the first to be made on the results of a series of investigations.

METHODS

Six lots of soil *A*, *B*, *C*, *D*, *E* and *F*, were collected. The first three lots were taken in November and the fourth in December, 1915, the fifth in January and the sixth in March, 1916.

Trenches were dug varying from 12 to 34 inches in depth, one trench for each lot. Soil samples were collected from the upper side of undercuts in the sides of the trenches at intervals of about 2 inches, from the surface of the ground down to the bottom of the trench. Lot *A* was taken from the grassland about 10 feet north of the northwest corner of the nursery. Lot *B* also came from the grassland 100 feet north of the northwest corner of the nursery. Lot *C* was taken from a seed-bed of *Pinus resinosa*, planted in 1914 in which all the seedlings had "damped off." It was neither planted nor cultivated in 1915 and contained no green plants. Lot *D* consisted of samples taken no deeper than 12 inches from a white pine grove several miles from the garden. *E* was collected in a

[6] Werkenthin, Frederick C. Fungous flora of Texas soils. Phytopath. 6: 241-253. 1916.

[7] In the preliminary experiments no attempt was made to determine the species of *Fusarium*. Consequently the term *Fusarium* as used in this paper is to be taken in the collective generic sense.

portion of a transplant bed which was made in 1914 and in which the trees had died from drought. This plot although cultivated in the season of 1915 contained no seed plants of any sort. Lot *F* was taken about 4 feet from *E* in the same sort of soil.

In the digging of the various trenches no precautions were taken against contamination from the air with the exception that the collections were made as soon as possible after the earth was exposed so that the sterilized boxes in which the soil was placed were not open to the air any longer than was absolutely necessary.

TABLE 1

Showing depths at which samples were collected and in which Fusarium occurred

A 9N15 Grassland	B 12N15 Grassland	C 23N15 Seed Bed	D 5D15 White-Pine Grove	E 24Ja16 Transplant Bed	F 15Mr16 Transplant Bed
Surface	Surface	Surface*	Surface	Surface	Surface
2 inches*	3 inches*	1 inch*			
3	5	2 inches	2 inches		2 inches*
5	6	3*	4	2 inches*	4*
8	8	4	6	4	6*
10	10	6	8	6	8*
12	11	8	10	8	10*
14	13	10*	12	10	12*
16	14	12		12*	14*
20*	16	14		14	16*
24	18	16		16*	18*
	20	18		20	20*
	22	20		24	22*
	24	22		28	24*
	26	24		34	26*
		26			
		28			
		30			

* Samples in which *Fusarium* occurred.

Two kinds of culture media were used, oatmeal agar and soil agar, the former on the whole appearing to give the better results. The soil agar was made according to the second formula described by Jensen. Although this medium was very satisfactory there was apparently too little difference in the reaction of the fungi toward it to pay for the additional time in preparing it. Practically the only advantage was that a more rosy color was produced by the *Fusarium* on the soil agar than on the oatmeal agar.

The cultures were made in the following manner: about 1 gram of soil from each sample was shaken up with 100 cc. of distilled water in a steril-

ized bottle. With a sterilized pipette 1 cc. of the solution was taken from each bottle, added to 10 cc. of melted agar and plated. Two plates were usually made from each bottle. The samples of lots *E* and *F* were placed in sterilized glass jars and thoroughly shaken to mix the soil well before making the solutions. At first the solutions were allowed to settle five minutes before pipetting off, but in *E* and *F* the 1 cc. was taken immediately after the solution had been well shaken. There was practically no difference in the number of colonies produced by the two methods. When fungous colonies appeared in the plates isolations were made in the usual manner for obtaining pure cultures.

RESULTS

As was to be expected a great variety of fungi developed in culture. *Fusarium* was found in the cultures from the soil taken both from the nursery and the grassland; in the nursery *Fusarium* occurred practically at all depths examined, not in all lots, from the surface to a depth of 24 inches, while in the grassland it was found at only three depths, 2, 3 and 20 inches. The finding of *Fusarium* in the cultures made from the soil of lot *A* taken at 20 inches from the grassland may have been the result of contamination although every precaution was taken to prevent it.

In lot *A* *Fusarium* was found at 2 and 20 inches below the surface; in *B* at 3 inches below; lot *C*, at the surface, 1, 3, and 10 inches. Micro- and macrospores were found in lots *A*, *B* and *C*. No fungi which are known to cause "damping-off" were found in lot *D*. In *E*, *Fusarium* occurred at 2, 12 and 16 inches and in these cultures many chlamydospores were observed as well as micro- and macrospores. *Fusarium* was found in lot *F* at every depth examined from 2 inches down to 24 inches, micro- and macrospores being most abundant, and chlamydospores fewer than in the cultures from lot *E*.

A possible explanation for the occurrence of *Fusarium* at the lower depths is that it may follow down living roots of such plants as clover and grasses as a parasite or the dead roots as a saprophyte. Sackett[8] believes that the character of the soil influences the depths at which bacteria occur; possibly the same is true of fungi. In arable soil Sackett finds that bacteria occur most abundantly in the first 7 or 8 inches, while in soil whose texture is loose and open they are found at lower depths. In irrigated soil he states that bacteria are carried down to a depth of 8 to 10 feet by irrigation water and alfalfa roots, and that in compact soil the lower limit is much nearer the surface.

[8] Sackett, W. G. Some soil changes produced by microorganisms. Colorado Agr. Exp. Sta. Bul. 196. 1914.

Moreover, it is probable that other agencies such as spiders, earthworms and larvae of May-beetles and other insects play a considerable part in the distribution of fungi in the soil, since spores may be carried down on their bodies or may be washed down through their tunnels from above. Earthworms and larvae of insects were found at the lowest depths at which soil was taken and cultures made from the contents of the alimentary canals of two earthworms revealed the presence of *Fusarium*.

Bolley[9] believes that a certain amount of moisture is necessary for a large development of certain fungi in the soil and mentions drainage water as a means of distribution.

Various investigators consider climate an important factor in connection with soil fungi.

Beckwith thinks it is possible that climatic conditions may influence "the mycological floras both saprophytic and parasitic."

Conn[10] finds more bacteria present in frozen soil than in unfrozen soil and points out the need of investigation along the line of seasonal variation among soil bacteria.

The results obtained by the writer also seem to indicate a possible seasonal variation of fungi since *Fusarium* occurred at so many more depths in March than during the previous winter months. It is also evident that what is true for bacteria may not necessarily be true for fungi as more *Fusarium* was found in unfrozen than in frozen soil. The question of seasonal variation of soil fungi is receiving further investigation which is to be reported later.

SUMMARY

1. Preliminary investigations to determine the depth to which *Fusarium* is present in soil indicate that *Fusarium* occurred to a depth of 24 inches in the nursery soil.

2. *Fusarium* occurred in more samples of soil from the nursery than from the grassland.

3. *Fusarium* appeared in culture from more samples in March than in the previous winter months indicating a possible seasonal variation.

[9] Bolley, H. L. Flax wilt and flax sick soil. North Dakota Agr. Exp. Sta. Bul. 50. 1901.

[10] Conn, H. Joel. Bacteria in frozen soil. New York (Geneva) Agr. Exp. Sta. Tech. Bul. 35. 1914.

BRIEFER ARTICLES

NOTES ON WOOD-DESTROYING FUNGI WHICH GROW ON BOTH CONIFEROUS AND DECIDUOUS TREES. II.

JAMES R. WEIR

Since the appearance of number I of this series,[1] additional collections of fungi growing on the wood of both coniferous and deciduous trees have been made. Correspondents have also contributed several interesting specimens with notes on host, range, and general habits of growth.

The mere listing of unreported hosts for saprophytic wood-destroying fungi which may occur on any woody substratum in the absence of the typical or common host is in most cases of mycological interest only, provided the species are correctly interpreted and a nomenclature of recognized standing employed. The preparation of such lists possibly serves a useful purpose, if much material is handled, in that it brings the collector to the realization that a new species does not lurk in every unusual form encountered. A thorough understanding, on the other hand, of the host relations of wound parasites is of great economic importance, for on this may be based plans for the proper silvicultural management of mixed forests. It is also of interest to note the occurrence on coniferous wood of fungi commonly found on the wood of dicotyledoneous trees which, although of no practical importance so far as wound parasites are concerned, may be so considered with respect to the destruction of fallen timber. It is to be noticed that a greater number of fungi commonly associated with the wood of deciduous trees is found on coniferous wood than is the reverse. This is particularly true in Western United States where great bodies of pure coniferous forests exist.

Dædalea confragosa (Bolt.) Fr. on branches of *Abies grandis*, Bellingham, Washington. Owing to the fact that the fungus is so closely associated with deciduous trees, especially willows and birches, its discovery on coniferous wood is surprising. The fungus was abundant on its usual hosts at Bellingham. The specimen collected is of the form known as *Trametes rubescens* (A. & S.).

Dædalea unicolor (Bull.) Fr. on fallen branches of *Abies lasiocarpa*, Priest River, Idaho. Semi-stipitate form but otherwise typical.

Irpex lacteus Fr. on *Betula occidentalis*, Priest River, Idaho. Speci-

[1] Weir, James R. Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. I. Phytopath. 4:271-276. 1914.

men collected and contributed by J. Huot. Fungus usually occurs in this region on Tsuga, Abies, and Picea.

Polyporus albellus Peck, a common birch wood fungus in the Northwest, has been collected once at Laclede, Idaho, on dead branches of *Abies grandis*.

Polyporus elegans (Bull.) Fr., usually on soft wood of deciduous trees, collected on branches of *Tsuga heterophylla*, Priest River, Idaho.

Polystictus abietinus (Dick.) Fr., small specimens on *Populus trichocarpa*. Typical.

Schizophyllum commune Fr. on *Tsuga heterophylla*, Seattle, Washington. Specimen contributed by Dr. J. W. Hotson.

Trametes carnea (Nees) Cooke, on fallen trunk of *Arbutus menziesii*, Grants Pass, Oregon. One small sporophore. Species is usually zonate. Collection has no surface markings but otherwise typical.

Trametes heteromorpha (Fr.) (*Lenzites heteromorpha Fr.*) (*Daedalea heteromorpha* Fr.) This plant usually grows on coniferous wood but is found occasionally on Alnus. Lloyd[1] recently described a new species (*Trametes lacerata*) collected on Alnus which probably belongs here. The pores of *Trametes heteromorpha* are very variable. It is very seldom a true Lenzites and is a better Trametes.

Trametes hispida Bagl. on *Pseudotsuga taxifolia*. Contributed by Dr. J. W. Hotson, Seattle, Washington. Very unusual host. In America, practically confined to Populus and Salix. Collected by the writer in Europe (Bavaria) on Fraxinus. Other collections by the writer in Bavaria and Serbia are closer to the American plant known as *Trametes peckii* Kalch. than any European material so far examined. In view of the fact that the European plant has usually smaller pores, is thinner, and of a distinctive brown color, it may cause less confusion if the two forms are held distinct. American plants in the writer's herbarium referred by Peck to *Trametes trogii* Berk. more nearly represent the European plant in this country.

Trametes serialis Fr. is usually found on coniferous wood. Specimens in the writer's herbarium are labeled "Collected on *Populus tremuloides*, Gila National Forest, New Mexico." The name of the collector is not given. The specimens have smaller pores than is usually the case.

Trametes variiformis Peck, on *Betula occidentalis*, Bellingham, Washington. This species may be considered a rare fungus even on coniferous wood.

OFFICE OF INVESTIGATION IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
MISSOULA, MONTANA

[1] Lloyd, C. G. Mycological Notes. No. 43, p. 604. 1916.

THE SPRAY METHOD OF APPLYING CONCENTRATED FORMALDEHYDE SOLUTION IN THE CONTROL OF OAT SMUT

R. J. HASKELL

The treatment of oat seed for the control of the smut diseases, caused by *Ustilago avenae* and *U. lævis* was placed on a commercial basis at least in North America when Bolley[1] announced the effectiveness of dilute formaldehyde solution. The sprinkling method as perfected by him and his contemporaries was adopted generally by American plant pathologists and is, at the present time, the common method of oat seed disinfection. In spite of the fact that it is one of the easiest, cheapest, and most efficient methods of plant disease control, it is not as widely employed as it should be, even with the great amount of publicity that has been given it. The wetting of the seed is objectionable to many.

Bolley[2] recognizes that a dry formaldehyde treatment would prove more popular with farmers and made some tests with this idea in mind. He obtained very encouraging results but has not yet proposed a method that can be put into practice on a large scale. Clinton[3] performed an experiment in the laboratory which indicated strongly that formaldehyde can be employed as a vapor in disinfecting oats to prevent smut, but nothing further seems to have been done by him looking to the elimination of the great volume of water called for in the original formula. Wheeler's work[4] with the influence of formaldehyde vapor on wheat seed and stinking smut also indicates the possibilities of this substanceas a smut preventive. Arthur's[5] rapid method of spraying falling grain in elevators with a 25 per cent formaldehyde solution is in reality a wet process rather than a dry one.

In view of the desirability of a dry treatment the writer has conducted

[1] Bolley, H. L. New studies upon the smuts of wheat, oats and barley. North Dakota Agr. Exp. Sta. Bul. 27: 109–162. 1897.

[2] Bolley, H. L. The prevention of smuts of cereal grains and prevention of potato scab. North Dakota Agr. Exp. Sta. Bul. 37: 363–379. 1899.

[3] Clinton, G. P. The smuts of Illinois agricultural plants. Illinois Agr. Exp. Sta. Bul. 57: 289–350. 1900.

[4] Wheeler, W. A. Preliminary experiments with vapor treatments for the prevention of the stinking smut of wheat. South Dakota Agr. Exp. Sta. Bul. 89: 1–19. 1904.

[5] Arthur, J. C. Rapid method of removing smut from seed oats. Indiana Agr. Exp. Sta. Bul. 103: 257–264. 1905.

laboratory and field experiments covering a period of four years, with the use of concentrated solutions of formaldehyde.

As a result of the investigation it has been found when one pint of undiluted 40 per cent formaldehyde solution is well distributed through fifty bushels of viable oats seed, which are then covered for five hours, that the germination of the oats is not impaired and the smut disease is controlled.

The amount of formaldehyde gas given off in evaporation is great enough to penetrate through the pile and to destroy all smut spores. On the other hand, the small amount of concentrated solution applied as a spray is insufficient in quantity to wet the seed to such an extent as to permit any absorption and consequent injury. In fact it often happens that seed thus treated germinate quicker and more vigorously than those remaining untreated. This may indicate that the formaldehyde vapor has a stimulating influence on germination comparable to that resulting from etherization, or there is a possibility that the phenomenon may be explained by the fact that the treatment frees the seed from certain semiparasitic and injurious organisms that may be associated with it.

When this method was first put into practice it was found that one pint of formaldehyde solution was hardly sufficient to satisfactorily cover fifty bushels of oats. Subsequent tests brought out the fact that the commercial 40 per cent solution could be diluted with water to one-half strength and then applied at the rate of one quart to fifty bushels of seed with better results.

The method as revised and as at present recommended may be briefly outlined as follows: As the seed is being shoveled from one pile to another each shovelful is sprayed with a solution consisting of one part of 40 per cent liquor of formaldehyde and one part of water. This solution is used at the rate of one quart to fifty bushels of seed. A small quart sprayer is a convenient one to use for the purpose. After the oats are all treated in this way they are piled in a heap and covered with blankets, canvas, or sacks to confine the vapor. At the end of five hours the seed may be uncovered and planted. As formaldehyde vapor acts as an irritant to the mucous membrane of the eyes, nose, and throat, the sprayer should be held down close to the pile and a circulation of air should be provided.

The conclusions stated above as to the effectiveness of this method are based on three personally conducted field experiments in two different seasons, and nineteen cooperative experiments, all of which were accompanied by germination tests for seed viability. In addition to this, to insure that there need be no hesitation whatever in recommending the treatment to farmers, it should be stated that numerous favorable testimonials have been received from those who have tried the method.

The field experiments were carefully checked, there being in one case a control for every two trial plots. All precautions were taken to avoid recontamination of the seed after treatment. These precautions included the use of clean containers, disinfection of the drill, and a consideration of the order of planting the different lots of grain. In all germination tests from one to two hundred seeds were used, and in recording the percentage of smut two or three thousand panicles were counted in different parts of each plot. The amount of smut in some of the checks ran as high as 25 per cent while that in the plots of treated seed was negligible.

In the cooperative experiments the amounts of treated seed varied from two to thirty bushels. In these cases the treatment was supervised or made by farm bureau agents or other competent persons. Treated and untreated seed were in all cases sent to the writer and tested by him for germination in the laboratory. Suitable checks were left in all cooperative fields and the percentage of smut in them determined in most instances by the cooperators themselves. Some of them reported as high as 25 per cent smutted heads with practical freedom from smut in treated plots.

The chief advantage of the dry method over that in which the 40 per cent solution of formaldehyde is sprinkled at the rate of one pint to forty gallons of water lies in the fact that the seed is not wet. This makes it possible to drill immediately after the disinfection is completed, a very important item when weather conditions are considered. The oats do not swell nor do they cause trouble by sticking in the drill. The operation is simpler than that of sprinkling, and the treatment is effective and non-injurious to the seed. Furthermore seed disinfected in this way can be kept several weeks after treatment without danger of deterioration. The method has met with favor in practically all cases where it has been tried.

CORNELL UNIVERSITY
ITHACA, NEW YORK

REVIEWS

Intoxicating Bread. N. A. Naumov. P'iany Khlieb (Intoxicating Bread). Trudy Biuro po Mik. i Fitopat. No. 12, pp. 1–216, pls. I–VIII, Petrograd, 1916.

A. Pomasski. Ob Izmienen. Khim. Sostava Rzhi pod Vliian. Zhizn. Niekot. Form Fusarium (Chemical Changes in Rye Due to the Activities of Certain Forms of Fusarium). Soobshch. Biuro po Chastn. Rasten. No. 1 (1916), pp. 1–32, Petrograd, 1916.

Considerable attention has been given in recent years by the Russian pathologists to an epiphytotic of cereals, known locally as drunk bread or intoxicating bread, ascribed to certain species of Fusarium. It affects rye, wheat, barley and oats, the maximum infection observed being 88 per cent on barley in 1912. The general appearance of the affected cereals in the field is practically identical in all varieties, a pale green color when young, smaller straw and heads, pink to red and orange-red sporodochia, and, in isolated cases, perithecia at the bases of the stems or in the nodes and on the sheaths. The affected grain acquires toxic qualities which are subsequently communicated to the flour and the bread. When these latter are used by men and animals typical symptoms of poisoning result, i.e., headache, general weakness, and frequently nausea and vomiting.

The disease is known both in European and Asiatic Russia, but with respect to its repeatedness, vigor, and proportions the Primorski region of Eastern Siberia appears to be classical.[1] It was recognized there long ago by the Chinese farmers by the surface film on the affected grains, called *mi-chun*. Russian colonization of this region began in 1862, and first mention of the disease by a scientist was made by Palchevski in 1882. Since then a number of persons, including mycologists, pathologists, and physicians have been working on this phenomenon. *F. roseum* almost invariably occurred in cultures made from the diseased cereals, and O. E. Gabrilovitch in 1906 demonstrated the toxicity of this fungus. Hypodermic injections have been made into frogs with the extract from pure cultures of the Fusarium with the result that the latter died within three to four hours after inoculation. The toxin was supposed to be a nitrogenous glucoside.

Several years ago the Bureau of Mycology and Phytopathology of Pe-

[1] Of the other countries it has been reported in Sweden and France (Prillieux and Delacroix, Maladies des plantes).

trograd determined to make a thorough investigation of this trouble and formulated two distinct problems: (1) myco-pathological, isolation and exhaustive study of the causal organism with possible reference to the control measures, and (2) biochemical, a study of the chemical changes which take place in the affected grains and the isolation of the active toxin.

The first problem was taken by N. A. Naumov, who just recently published a very interesting report on his work. He submits evidence that the phenomenon "drunk bread" may be caused by either of two species of Fusarium, namely, *F. roseum* Link. (also its ascus stage *Gibberella saubinetti* Sacc.) and *F. subulatum* App. & Woll. The chief distinctive feature of the disease outside of chemical and physiological peculiarities, according to the author, is the presence of the mycelium inside of the grain tissues, which can be proven either microscopically or by means of germinating the seed. Red or pink color of the grain and presence of sporodochia or perithecia cannot serve as leading characters, as they sometimes may be absent even when the grain is plainly affected. In the stems grown from the diseased seed the mycelium has been found nesting in the tissues and penetrating the cells and intercellular spaces, but it is absent in the vascular bundles as well as in the primary meristem. No anatomical changes were observed in the infested stems, but the seeds suffer severely, as starch is dissolved and frequently the embryo is mummified. The disease is communicated to the new crops through infected seed or infected soil, or from an adjoining infested field. The germination of ascospores and conidia takes place in neutral, slightly acid or slightly alkaline media, a slight alkalinity being tolerated better than the corresponding acidity. An excess of moisture in cultures favors the growth of mycelium but is unfavorable for the production of conidia. The best media were found to be rye in grains, heads of wheat, heads of rye, and milk. The minimum temperature for germination of conidia is 4°C., optimum 28°C., and maximum 32°C.; for ascospores, 8°C., 30°C., and 32°C. respectively. Optimum growth of mycelium occurs at 30°C.; there is no growth below 3°C. or above 33°C. The best formation of the conidia and perithecia is at 20°C. Change in temperature is more beneficial for fruiting than injurious. Heating of ascospores for twenty-four hours at 65°C. destroys their ability to germinate. The mycelium completely loses its vitality in grains if the latter are stored under ordinary conditions for a period of three years. Heating rye for twenty-four hours (and up to three days) at 66°C. and wheat, oats and barley at 60°C. does not affect the vitality of the seed, but kills the infesting mycelium. This may prove to be the chief method of treatment. Other control measures suggested are, (1) selection of healthy seed, (2) two to four years crop rotation with root

crops and legumes as intermediate crops, (3) threshing at once after harvesting and possibly treatment of the portion intended for seed with 0.5 to 1 per cent copper sulfate to prevent spread of the disease in storage (in this case the seed should be dried before putting in granaries), and (4) general sanitation and disinfection of sacks and implements. Positive results were obtained in artificial inoculations when soil, shoots, and heads were infected with the conidia and the ascospores and when soil and shoots were infected with the mycelium. Considerable attention is given by the author also to the taxonomic side of the problem.

The biochemical part of the problem has been studied by A. Tomaszki, and a preliminary paper dealing with this work has been already published. In it the author shows the chemical changes which take place in rye as the result of the activities of *F. roseum* and *F. subulatum*. He finds that these activities lead to a marked loss of dry substances, which decrease to 67.3 per cent in ten days and to 25.1 per cent in two months. This loss takes place chiefly at the expense of starch and proteins. Decrease of starch in one month of decomposition reaches from 61 to 80 per cent and in two months from 86.5 to 89.5 per cent of the original quantity. Decrease of the general amount of nitrogen in two-month's cultures equals 12 to 16 per cent. Both forms of Fusarium affect rye in more or less the same way. Other changes as well as the products of decomposition are to a certain extent determined. A detailed study of these products and, particularly, isolation and study of the toxic substance of "drunk bread" will constitute a subject for the further studies of the author.

MICHAEL SHAPOVALOV

Manual of Fruit Diseases. By Lex R. Hesler and Herbert Hice Whetzel. xx + 462 pp., 126 illustrations. Published by the Macmillan Company, New York, 1917.

The book is one of the publisher's series of Rural Manuals and has been written with the definite aim of giving the fruit grower a working knowledge of fruit diseases. The treatment of the subject is from the field rather than from the laboratory standpoint.

The arrangement is alphabetical by crops, the diseases under each crop being approximately in the order of their economic importance. The fruit crops included are apple, apricot, blackberry, cherry, cranberry, currant, gooseberry, grape, peach, pear, plum, quince, raspberry and strawberry. There is a concise chapter on the preparation and application of fungicides. Diseases common to more than one host are discussed fully under the most important one and listed under others with such special comment as may be warranted.

The discussion of each disease of major importance includes its general history, geographical range with special reference to regional occurrence in the United States, the nature and extent of losses caused, general effects on the host and diagnostic symptoms, relative susceptibilities of host varieties, the cause of the disease with discussion of contributory factors, the life histories of parasites with particular reference to seasonal development and conditions influencing outbreaks, control measures with emphasis on the rational basis for such measures as well as on the practical details of their application. Well selected illustrations give typical gross features of the diseases. Minor diseases are accorded unusually full discussion. Reference lists under each disease direct the reader to the more important readily available publications, mainly American.

Out of consideration for the grower technical details of taxonomy, morphology and histology have been omitted; and the use of technical terms, while not by any means avoided, has been reduced to the bare requirements of concise statement. A glossary gives brief definitions of many of these terms.

The style is not the unfortunate kind that sometimes characterizes the popular treatment of a technical subject. It has the directness, clearness, exactness and suitableness expected from scientists who are also teachers. The book can be read by either layman or specialist without mental fatigue.

Any student of plant pathology will find the book helpful for reference. With the usual laboratory work to supply a proper morphological and histological framework, the only important limitation to its usefulness as a textbook in general plant pathology courses is the restriction of its scope to a particular group of diseases.

A few defects will be apparent to the critical grower or pathologist. These cannot be pointed out in detail in this review; they would require in the main but slight revision for remedying—a shifting of emphasis here and there, a fuller explanation and illustration of certain technicalities, the correction of occasional lapses from the usual excellence of treatment, the addition at times of another desirable reference, the inclusion as synonyms of imperfect stage names of fungi that have been known commonly by such names, the amplification or modification of details in certain control procedures, the giving with greater reserve of recommendations for control by dusting with sulphur, a broadening geographically of the intimate statements about orchard conditions Attention may be called to differences in the usage of common names from the provisional list prepared by the committee of the American Phytopathological Society. For apple, apricot and blackberry, thirty of the diseases listed by the committee are treated in the Manual; twelve of these are under preferred common names different from the committee's recommendations; and in

ten cases out of the twelve the committee's names do not appear in the unusually complete index.

The volume is a distinctly helpful advance in the somewhat slow evolution of a book literature of American plant pathology.

H. R. FULTON

PHYTOPATHOLOGICAL NOTES

Method for photographing culture plates. A most satisfactory method of making photographic reproductions of bacterial colonies on culture plates has been found as follows:

The petri dish is placed directly upon the sensitive side of the photographic paper and the cover of the dish removed. The culture plate is

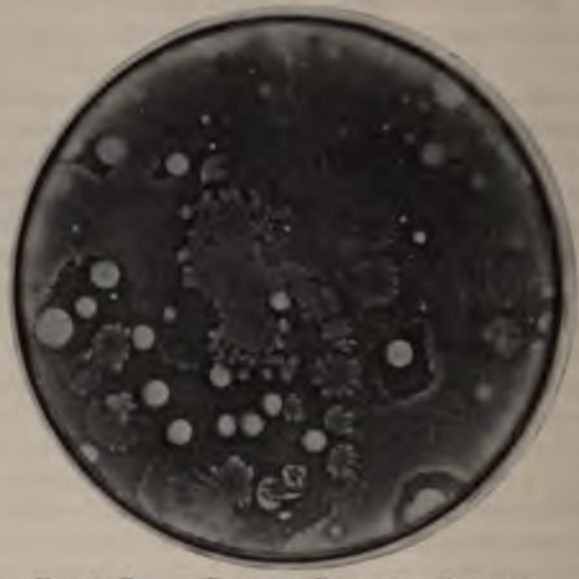


FIG. 1. DIRECT PRINT OF BACTERIAL COLONIES

then exposed directly beneath a light for a few seconds and the print developed. The writer uses Azo, glossy Hard C, and a frosted Mazda 40-watt light with exposure made on a shelf about twelve inches below the light.

The advantages of this method are plainly to be seen. First, no negatives are required; second, the process is rapid and many prints can be made in a very short time; third, considerable expense in materials and the operator's time is saved and uniform results are obtained. Also a saving of valuable filing space, occupied by photographic plates that are not always satisfactory, is accomplished.

W. S. FIELDS

Methods for the differentiation of pathogenic fungi in the tissues of the host. In the investigation of problems in plant pathology it is frequently desirable to be able to locate and trace the mycelium of a pathogenic fungus in sections of the tissues of the host plant. To this end recourse is usually had to some method of staining by which the hyphae may be differentiated from the cells of the host. For this purpose several special stains and methods have been devised, notable among which are Durand's[1] and "Pianeze III b," as described by Vaughn.[2] It is said that these stains, while producing excellent differential effects with some material, can not always be depended upon to define sharply all mycelium regardless of species and fungus and host.

The writer has been fortunate in the application of two methods which, so far as he is able to determine, have not been described heretofore in this connection.[3] These methods are stated here in the hope that they may be effective when others fail to give the desired results.

The first method consists of a methylene blue-clove oil-eosin combination[4] applied to paraffin or free-hand sections as follows:

Having dehydrated the sections in absolute alcohol, stain 3 to 30 minutes, or longer, in a filtered, saturated solution of methylene blue in absolute alcohol; rinse off excess of stain in water; dehydrate rapidly by flooding inclined slide with a few drops of absolute alcohol; drain immediately and flood sections with a few drops of a filtered saturated solution of eosin or erythrosin in clove oil; watch extraction of methylene blue and taking of counterstain with microscope until desired effect is produced (which should be in from 1 to 10 minutes); flood inclined slide with a mixture of equal parts of absolute alcohol and xylol to remove excess of clove oil stain and prevent formation of an objectionable red precipitate, and place in xylol to clear.

This method, judiciously followed, produces a deep blue stain in the protoplasm of the fungus and a lighter blue in the protoplasm of the host cells, while the cellulose cell walls of the host take a deep pink or red, as well as the walls of the hyphae. Hence, if a piece of mycelium traverses or lies in a cellulose host cell wall, its course is shown by the blue stain

[1] Durand, E. J. The differential staining of intercellular mycelium. Phytopath. 1: 129–130. 1911.

[2] Vaughn, R. E. A method for the differential staining of fungous and host cells. Ann. Missouri Bot. Gard. 1: 241–242. 1914.

[3] Dr. Neil E. Stevens and Dr. Lon A. Hawkins have been kind enough to mention the first of these methods in the Jour. of Agr. Research 6: 362, and 635 (footnote), 1916, respectively.

[4] Zimmerman, A. Botanical Microtechnique (Translated by Humphrey, 1893). On p. 185 the use of eosin in clove oil as a counter stain in Gram's gentian violet method is mentioned.

of its protoplasmic contents, while if it passes through protoplasm of the host, its path is marked by the pink-stained walls of the hypha and its frequently more dense protoplasm. The presence of fungi in lignified elements of the host is shown by the pink hyphal walls against the blue background of the lignified walls.

It has been found that the methylene blue sometimes washes out too easily from the protoplasm of the fungus. Mordanting the sections in an aqueous solution of tannic acid (about 10 per cent) for half an hour, washing in water, and dehydrating to absolute alcohol before applying the methylene blue has proved to be of considerable advantage in holding the stain in the protoplasm of the fungus.

The second method, anilin water, safranin and clove oil—lichtgrun[5] may be described as follows: Stain sections 1 to 12 hours in anilin water safranin;[6] rinse in water; dehydrate by giving slide a quick dip into each of 70 per cent, 95 per cent and absolute alcohol; flood sections immediately with a few drops of a filtered, saturated solution of lichtgrün in clove oil (twice rectified); proceed as for first method.

The differentiation of the fungus in this case depends upon the deep red stain of its protoplasmic contents against a light green in the cellulose cell walls of the host or the light green hyphal walls against the pink or red protoplasm or lignified cell walls of the host.

The secret of success with these stains seems to lie in retaining as much as possible of the first stain in the protoplasm of the fungus before the application of the clove oil stain. This latter extracts the first stain in a differential manner and at the same time has a counter-staining effect, hence the necessity of carefully watching the first few slides of a series from a lot of material in order to determine the time required for each operation. While slides stained by these methods have been prepared for over a year and still show good differentiation, the stains will doubtless fade in time and therefore may not be desirable where it is planned to preserve the preparations indefinitely. The advantages of the methods lie in the facts that, in certain cases at least, they produce excellent differentiation, that they are easy to apply, and that they are relatively simple and inexpensive in composition.

Both of these methods have been found effective in connection with

[5] This stain was first brought to the writer's attention by Mr. E. G. Arthberger of the Office of Agricultural Technology, Bureau of Plant Industry, who was using safranin and an aqueous solution of lichtgrun with partial success for the differentiation of fungi in host plant tissues. Following a demonstration of the clove oil method he promptly discarded the aqueous solution method in favor of the one herein described.

[6] General Formula. Chamberlain, C. J. Methods in Plant Histology, second edition 1905, p. 38.

Botrytis (*cinerea?*) and *Rhizopus* (*nigricans?*) in strawberry fruits,[7] *Pythium debaryanum* in Irish potato tubers,[8] *Fusarium* sp. in tobacco stems, the aecial stage of crown rust of oats in leaves of *Rhamnus cathartica*, and several unidentified organisms in the roots of various plants.

CHARLES S. RIDGWAY

Development of blister rust aecia on white pines after they had been cut down. A small plantation of badly diseased pines set out for seven years at Cookstown, Ontario, was cut down between November 9 and 15, 1916. The trees, many of which were two to three inches in diameter were left lying on the ground or more or less loosely piled until the spring of 1917. On May 10, 1917, the field man assigned to this district reported that fresh aecia were appearing around the old blister areas in these pines, which had not meanwhile been disturbed. A visit was at once made, which confirmed his observations. Where the trunks still retained some moisture new aecia were pushing out around the old blister areas. They were apparently as numerous as usual, though rather smaller than normal in size. In the drier stems, aecial development had occurred under the bark, where spores were being produced in immense numbers in irregular masses, forming an almost complete layer for 6 inches or more beneath the bark. This layer-like mass of spores extended under the old infections as well as beneath the bark of adjacent tissue, which had up to this time borne no blisters. Since about 25 per cent of the stem cankers were thus producing their usual crop of blister spores in this case, the menace that may arise from pines not completely destroyed, is clearly apparent.

During the course of field observations upon an area of diseased pines at Kittery Point, Maine, trees eight to thirty-five years old with basal diameters 2 to 8 inches were cut down in November and December, 1916. Many cankers were collected for exhibition material and the remaining slash left on the ground over winter. On May 2, 1917, mature blisters were found on stems and branches of various sizes which were cut November 18. Subsequent observations showed that aeciospores were produced on approximately 60 per cent of the infected stems and branches Normal blisters were formed in many instances, and in several cases where the cut ends of stems were placed in running water spore production continued until June 18. On infected stems 3 to 8 inches in diameter which began to dry out before blisters showed through the bark, spores were produced in irregular masses under the hardened bark. Some of these

[7] Stevens, Neil E. loc. cit.
[8] Hawkins, Lon A. loc. cit.

spore masses retained normal color until June 25, after which the orange color was lost and the spores appeared in light grayish masses when the bark covering was removed. On June 3, 1917, a sporulating canker on a limb 2 inches in diameter which had remained on the ground since November was sent to Washington. Germination tests made of the spores at Washington, June 6, gave positive results. Spores from this canker were used for inoculating a plant of *Ribes nigrum* under greenhouse conditions on June 8, and normal uredinia were subsequently produced. On March 20, 1917, freshly cut infected branches and one section of a stem 4.5 inches in diameter were received at Washington. The branches were set in water and blisters appeared in a few days. The stem section was about three feet long. All side branches were closely trimmed off and it was set on top a case near the ceiling of the laboratory. About May 1 blisters were pushing through the bark in spite of the drying effect of the heat.

These findings show that wherever diseased pines are removed the brush and affected parts of the trunks must be carefully collected and burned in order to make the work effective.

W. A. MCCUBBIN AND G. G. POSEY

Control of lettuce rot. A soft rot of lettuce of the type described by Miss Brown[1] and ascribed to *Bacterium viridilividum*, has been seen in many fields in Michigan doing enormous damage to the crop. What appears to be the same disease has been found in the market at Chicago in lettuce from New York as well as from other states. Carload after carload of lettuce arrives in the great markets almost a total loss. Fifty per cent loss is not uncommon in the field, and this, coupled with the loss which occurs in transit, makes the growing of head lettuce in some seasons very precarious.

The disease starts first at the tips of the leaves, probably in the dead areas which come at the edges of the older leaves. These areas shrivel and curl and are marked off from the healthy tissue by a definite line. With the inner leaves the rot progresses very deep into the head, turning their tender leaves into a gluey slime. The deep involvement of the inner leaves and the comparatively small amount of rot of the outer leaves is characterisic of the early stages of this trouble. Eventually the whole head rots.

The purpose of this note is to call attention to the remarkable control of the disease obtained by spraying diseased plants with formaldehyde. 1

[1] Brown Nellie A. A bacterial disease of lettuce. Jour. Agr. Research 4: 475–478. 1915.

pint to 30 gallons of water. In tests in 1916, at Bay Port, Michigan, the disease was checked by this treatment in fields which were about to be given up as a total loss. The weather had, however, become very cool, hence the relation of the formaldehyde spray was not definitely proved.

The present season the rot began to be serious during an extremely wet and cold period in July. Spraying with formaldehyde has completely checked the trouble. In an adjoining field, the owner, seeing the success of the formaldehyde treatment, sprayed a portion of his field. In the sprayed portion the disease is checked while the unsprayed field shows a high percentage of rotting. A third field in the neighborhood, unsprayed, is almost a total loss.

Attention is called to this control measure because the results of the trials during two seasons warrant extensive tests of treatment. It may be pointed out that we have here a method which differs from the ordinary protective spray, in that it seems actually to check disease already in progress. It is believed that this treatment will be found to have a wide range of adaptability in the treatment of diseases of this type.

EZRA LEVIN

LITERATURE ON PLANT DISEASES

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

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PHYTOPATHOLOGY

VOLUME VII NUMBER 6

DECEMBER 1917

A TWIG AND LEAF DISEASE OF KERRIA JAPONICA

V. B. STEWART

WITH SEVEN FIGURES IN THE TEXT

A fungous disease caused by a species of Cylindrosporium is commonly found on *Kerria japonica* DC. The disease not only results in premature defoliation but it also affects the shoots, often injuring the bushes to such an extent that they die during the winter. In one ornamental nursery observed by the writer the growing of *Kerria japonica* stock has been discontinued on account of this malady.

SYMPTOMS

The first sign of the disease on the leaves are small discolored areas which soon become reddish brown in color. These areas are irregular in outline and vary from one to four millimeters in diameter (fig. 1, *A*). Often several lesions become confluent, involving a considerable portion of the leaf. The center of the lesion is slightly raised and may appear as a white velvety pustule. Severely affected leaves turn yellow, shrivel and fall prematurely. There is no shot-hole effect produced on the leaves as in case of certain Cylindrosporium diseases of other plants.

The lesions on the shoots are circular, reddish brown to black in color and vary from one to several millimeters in diameter (fig. 1, *B*). Usually the lesion is slightly sunken with the central area somewhat raised and covered with a white mass of conidia. In old lesions portions of the cortical tissue may fall out leaving the woody tissue exposed. Often times the diseased areas are so abundant as to completely girdle the shoot, this being especially true in case of small plants in the nursery.

THE FUNGUS IN THE TWIGS

The disease was first observed on *K. japonica* twigs in November after the bushes were defoliated.[1] No spores were apparent in the lesions when

[1] The affected twigs were examined by Prof. F. C. Stewart of the Geneva Agricultural Experiment Station, who stated that the cause of the disease was a species of Cylindrosporium. Professor Stewart also informed the writer that on several different occasions he had received specimens of the disease.

examined but after placing twigs in a moist chamber for three days there was such an abundant production of spores that the masses of them were apparent to the unaided eye (fig. 2).

Cross-sections of the affected area on a shoot show a stromatic layer of mycelium which extends through the cortical tissue to the wood. The stroma may be several hundred microns in thickness. The cortical tissue is disorganized but apparently there is practically no breaking down of the individual cells, isolated host cells often being enclosed in the stroma. From the upper surface of the stromatic layer short conidiophores are developed which produce masses of conidia. With the accumulation of the conidia the epidermis is broken and the spores are exposed on the surface of the twig. The fruiting body is a typical acervulus and the spores are characteristic of the genus Cylindrosporium.

Conidial production on the twigs ceases in the autumn and the fungus remains dormant until the following spring at which time it again becomes active.

On April 8, 1916, diseased twigs which had been exposed to the weather throughout the winter, showed in the center of the lesions a definite, slightly raised, circular area which was somewhat darker in color and sharply outlined. Cross-sections of the lesions showed the development of new hyphal threads and, with the occurrence of warm, rainy weather, conidiophores were produced from the outer surface of the stroma. Conidia appeared about the time the new foliage developed on the bushes. Abundant spore production was observed also in old lesions on living *Kerria japonica* bushes which had been affected the previous season.

The spore-bearing hyphal threads in the stroma stain more intensely and have the appearance of ascogenous hyphae but in all of the material examined there has been no development of a sexual fruiting body on the twigs.

THE FUNGUS IN THE LEAF

The growth of the fungus in the leaf is like that of other species of Cylindrosporium. The stroma first consists of a layer of one or two cells and increases in thickness as the acervulus grows older. The conidia, which are borne on short conidiophores break the epidermis and accumulate as a white mass in the center of the lesion (fig. 2). Spore production continues throughout the summer. Acervuli may appear either on the upper or lower side of the leaf. The mycelium is intercellular and derives its nourishment by means of haustoria which penetrate the host cells. Apparently the disorganization of the tissue is mostly mechanical, since there is no evidence that the fungus produces an enzyme which breaks down the cell walls.

FIG. 1. COCCOMYCES KERRIÆ ON KERRIA JAPONICA

A, leaves from bushes artificially inoculated with ascospores of Coccomyces Kerriæ. Slightly enlarged; *B*, lesions of the disease on young shoots of Kerria japonica.

FIG. 2. CROSS-SECTION OF LEAF SHOWING THE ACERVULUS WITH CONIDIA. × 340

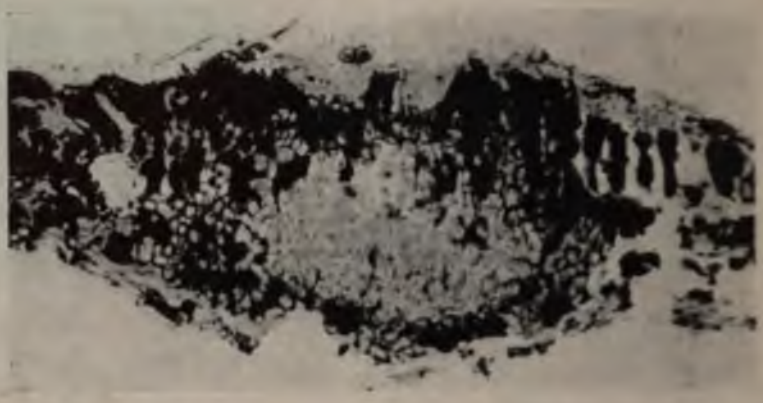

FIG. 3. THE EARLY DEVELOPMENT OF AN ASCOCARP OF COCCOMYCES KERRLÆ

The light-colored cells in the center are surrounded by the thick-walled pseudoparenchymatous tissue. × 340.

FIG. 4. CROSS-SECTION OF AN ASCOCARP OF C. KERRLÆ

The intensely stained ascogenous hyphae are in the central area. × 340.

DEVELOPMENT OF THE PERFECT STAGE

In the autumn the stroma of the fungus increases until it involves almost the entire thickness of the leaf. In cross-sections the inner portion of the stroma appears as thin-walled, light-staining, mycelial tissue surrounded by a denser pseudoparenchymatous layer (fig. 3). In the central area several more deeply-staining, hyphal coils are apparent. The upper end of each coil, which consists of several uninucleate cells extends toward

FIG. 5. A MATURE ASCOCARP OF C. KERRIÆ

This shows the asci and paraphyses. Another ascocarp is apparent on the opposite side of the leaf. × 340.

the surface of the stroma. Late in the autumn the hyphal coils disappear but portions of the coils have been observed in material examined as late as January 25.

Early in the spring the stroma in diseased leaves, which have been exposed throughout the winter, enlarges outward due to the development of erect, simple or branched paraphyses and soon afterwards ascogenous hyphae arise from the lower area of the stroma (fig. 4). The paraphyses rupture the covering over the stroma in a stellate manner exposing the asci (fig. 5) which become mature about the time new foliage appears on the host. When suitable moisture conditions are present the long slender

ascospores are ejected with force from the asci (fig. 6, *A*). No pore has been observed in the tip of the ascus.

The development of the ascocarp is similar to that of *Coccomyces hiemalis* described by Higgins[2] who discusses in detail the presence of hyphal coils and trichogyne-like bodies. In case of *C. hiemalis* the end of each hyphal coil extends above the surface of the stroma and terminates in a swollen trichogyne-like structure. The writer has made diligent search for the trichogyne-like structures in the fungus on *Kerria japonica*. Hyphal coils have been traced to the surface of the stroma, but no swollen structures have been seen which are comparable to the trichogynes of *C. hiemalis*. Numerous hyphal threads with swollen tips have been observed but it has not been possible to determine whether these were trichogynes or enlarged conidiophores. The organisms are similar in the fact that the production of filiform, curved, conidia (macroconidia) on the foliage ceases in late summer and in the same acervuli minute microconidia (spermatia) develop instead. The microconidia or spermatia of the fungus on Kerria measure about 2 by 0.5 μ being slightly smaller than those of *C. hiemalis*. Occasionally the spermatia are enclosed in definite pycnidia-like spermagonia which are about 40 μ in diameter. The spermagonia

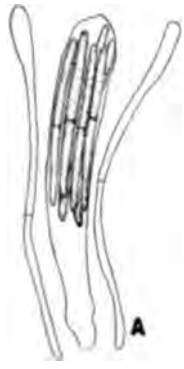

FIG. 6

FIG. 7

FIG. 6. ASCUS, PARAPHYSES AND ASCOSPORES OF *C. KERRIAE*

A, paraphyses and ascus with spores. × 525; *B*, germinating ascospores. × 600

FIG. 7. CONIDIA OF *COCCOMYCES KERRIAE*

One of the spores shows the development of a germ-tube. × 600.

[2] Higgins, B. B. Contribution to the life history and physiology of Cylindrosporium on stone fruits. Amer. Jour. Bot. 1: 145-173. 1914.

with spermatia have been observed on leaves of *Kerria japonica* exposed throughout the winter and even when the ascocarps are mature in the spring.

SYSTEMATIC POSITION

The ascocarp of the fungus herein discussed is similar to the forms included in the genus Coccomyces of the Phacidiales, but so far as the writer is aware no fungus of this genus has been described as affecting *Kerria japonica*. The name *Coccomyces Kerriæ*[1] is therefore proposed for the species and the following characterization given:

Coccomyces Kerriæ n. sp.

Ascogenous stage. Ascocarps embedded in tissue, usually on under side of leaf, scattered or somewhat aggregate, ovate, dark brown to black, 100 to 220 μ in diameter, at first closed but opening at maturity in a stellate manner; hymenium grayish; asci clavate, slightly papillate at apex, 55 to 74 by 11 to 13 μ; paraphyses simple or branched, septate, slightly swollen at tip; ascospores 8, hyalin, slender, slightly curved, continuous or once-septate, 33 to 48 by 2.8 to 4 μ.

Conidial stage. Spots numerous, reddish brown, 1 to 5 millimeters in diameter, often confluent; mycelium intercellular with haustoria which penetrate the host cells; acervuli scattered, amphigenous, subepidermal, finally breaking through and exposing the spores; conidia filiform, curved, hyalin, 1- to 2-septate, 40 to 76 by 3.2 to 4.8 μ. Spermatia very minute, hyalin, non-septate, 2 by 0.5 μ in old acervuli, occasionally in small pycnidia-like spermagonia.

Coccomyces Kerriæ n. sp.

Ascomatibus hypophyllis vel amphigenis, sparsis aut subaggregatis, punctiformibus, ovatis, negris, 100–220 μ lat., primum clausis, deinde in lacinias plures acutas dehiscentibus; disco griseo; ascis clavatis, apice leviter papillato, 55–74 x 11–13 μ; paraphysibus filiformibus, simplicibus aut ramosis, septatis, apice tumido; sporidiis 8, linearibus, leviter curvis, hyalinis, simplicibus aut 1-septatis, 33–48 x 2.8–4 μ.

Hab. In dejectis foliis Kerriæ japonicæ.

Status conidicus: maculis numerosis, minutis, rubrofuscis, 1–5 mm. lat., sæpe confluentibus; acervulis solitariis, amphigenis, subepidermicis, disciformibus; conidiis filiformibus, flexuosis, hyalinis, 1–2 septatis, 40–76 x 3.2–4.8 μ; sporulis minoribus auctumno hyalinis, continuis, minutissimis, 2 x 0.5 μ.

[1] Type material is deposited in the herbarium of the Department of Plant Pathology, Cornell University and in the herbarium of the New York Botanical Gardens.

CULTURAL STUDIES

The fungus is readily cultured on ordinary media such as potato agar, sterilized bean pods, or sterilized twigs of *Kerria japonica*. On June 2, 1917, an abundance of ascospores was obtained by inverting petri dishes containing a thin layer of agar over ascocarps which had been moistened. The ascospores were ejected with force and lodged on the agar a half centimeter or more above the ascocarps. Individual ascospores were transferred from the petri dishes to tubes of potato agar where the ascospores germinated (fig. 6, B) after twenty-four hours and developed cultures of the fungus.

The growth at first was slow and consisted of a thin grayish stroma of mycelium. On June 22 the stroma was about 4 or 5 millimeters in diameter, slightly raised and darker in the center. From the central area of the stroma conidia were produced similar to those found in lesions on the host plant. Portions of the mycelium were transferred on June 22 to test-tubes containing bean pods and twigs of *Kerria japonica* and a copious mycelial growth was obtained on both media. When the cultures were examined July 2 the older mycelium was turning black and pinkish gray masses of conidia were apparent. Only a small proportion of the conidia germinated when placed in water. However, five to ten per cent of the conidia showed germ-tubes after twenty-four hours (fig. 7) and the percentage of germination was slightly increased after forty-eight hours. As is characteristic of certain other species of Cylindrosporium, the conidia appear to lose their vitality very rapidly, in all the tests made of conidia from different sources only a small percentage of germination being obtained.

INOCULATION EXPERIMENTS

On May 28, 1917, ascospores which had been ejected to plates of agar, were placed on leaves of two healthy bushes of *Kerria japonica* which were growing in the greenhouse. The plants were then moved out-of-doors where they were exposed to a rainfall that lasted for sixty hours. Three other plants which were not inoculated were also placed outside and served as checks. When examined June 12 several of the inoculated leaves showed infection. Five days later conidia of Cylindrosporium were apparent in the lesions. The check plants remained healthy.

On May 29, 1917, isolated ascocarps of the fungus were crushed in water and the suspension of ascospores sprayed on the leaves of two bushes of *Kerria japonica* outside the greenhouse. Rainy weather prevailed for twenty-four hours after the inoculations were made. On June 12 numerous lesions of the disease were apparent on the inoculated leaves and an

occasional lesion was noticed also on the twigs. Two check plants which were not inoculated remained healthy.

On July 2, 1917, conidia which had developed in a culture from a single ascospore, were used for inoculating one bush of *Kerria japonica* growing in the greenhouse. A suspension of the spores in water was sprayed onto the leaves by means of an atomizer. Another plant was sprayed with water for a check. Both plants were placed in a moist chamber for thirty-six hours. On July 13 the characteristic lesions of the disease were apparent on the inoculated leaves and on July 18 conidia of the fungus were being produced. The check plant remained healthy.

The above experiment was repeated on July 10 and on July 21 conidia were observed in the lesions which developed in the inoculated leaves. It is to be noted, however, that only a relatively small number of lesions appeared on any of the inoculated plants where conidia were used. In spite of the fact that an abundance of conidia was sprayed onto the plants only a small proportion of them germinated and produced infection. In most cases there was an average of only one or two lesions on each leaf. Of plants inoculated with ascospores many of the leaves showed numerous infections. These results are in accord with the germination tests previously discussed.

Secondary infection occurred throughout the summer on the inoculated plants and in the autumn many of the shoots were severely affected and the plants were completely defoliated.

CONTROL

No carefully conducted experiments have been performed for the control of this disease, but preliminary tests made in 1916 indicate that a sulfur fungicide will prove effective in checking the malady. Bushes of *Kerria japonica* were growing in a nursery and severe infection had occurred before they were treated in 1916. However, four treatments during the summer greatly reduced the amount of infection and premature defoliation as compared to untreated bushes. One plat was treated with a dust mixture of ninety parts finely ground sulfur and ten parts powdered arsenate of lead. Another plat was sprayed with lime-sulfur solution 1 to 50 and the third plat was left untreated for check.

The production of conidia in the old lesions on the shoots early in the spring along with the liberation of the ascospores from the old leaves makes it exceedingly difficult to prevent the primary infections. This is especially true when weather conditions are favorable for the fungus.

CORNELL UNIVERSITY
ITHACA, NEW YORK

THE CROWN CANKER DISEASE OF ROSE

L. M. Massey

With Three Figures in the Text

On September 9, 1916, while visiting rose houses in the vicinity of Philadelphia the attention of the writer was called to a number of American Beauty plants affected with a hitherto unreported disease, to which the name crown canker has been given. The grower stated that he had had the disease under observation during the past four or five years, a few plants being affected each year and the disease being confined to a single house.

Subsequently plants affected with the crown canker disease have been received from eight growers, the states of Missouri, Pennsylvania, Indiana, Michigan, Massachusetts, and New York being represented. A Missouri grower observed the disease in 1916 on the varieties Hoosier Beauty and Ophelia growing on their own roots. A Massachusetts grower was of the opinion in 1916 that his entire stock of 60,000 or more plants was affected, and it is the opinion of the writer, after having examined his plants, that at least a very large percentage of them were diseased. During the four years prior to 1916 increasingly poor results were obtained by this grower who when interviewed in November 1916 was planning to destroy most of his plants, sterilize houses and soil, and begin anew with healthy plants.

Rose plants of the varieties Hoosier Beauty, Ophelia, Hadley, Russell, Sunburst, American Beauty, and many seedlings have been observed affected with the disease. Both grafted plants and those growing on their own roots are affected. It is questionable whether or not any variety is immune. Indications are that this may prove to be the most important disease of roses grown under glass. To date no record of the disease on out-of-doors plants has been made.

SYMPTOMS

Diseased plants are affected at the crown, usually just at the surface of the soil, the lesion in advanced cases frequently extending several inches above the soil. The writer has not determined to what extent the root systems are commonly affected. However, lesions have been observed near the tips of roots of four-years-old plants, and of several plants examined unquestionably the entire root system of each plant was affected.

The union of the scion and stock, and the area immediately above, is the most common point of attack.

The first indication of the disease is a slight discoloration of the bark. As the disease advances the color rapidly deepens to black and the tissue appears water-soaked (fig. 1, *A*, *C*). At first the lesions are irregular in outline with a somewhat sharply defined margin. Later as the affected area increases in size the blackened color of the diseased area is blended more with the healthy tissue. The lesions frequently encircle the stem. Soon cracks appear in the bark extending in to the wood (fig. 1, *B*). Later a swelling of the stem as from girdling occurs at and above the affected area, the cracks becoming deeper and more evident. In old lesions the black, water-soaked appearance is lost. Sometimes the stem is encircled by a shrunken area which contrasts sharply with the swollen area immediately above.

One very noticeable characteristic of this disease is the punky consistency of the diseased tissue, especially that affected under ground. When scraped, the bark, sapwood and frequently the roots appear punky and lifeless, not unçommonly in areas where no definite lesion is evident.

Suckers developing from the roots of diseased plants are usually spindling and yellow. They are commonly affected at the point of attachment to the main stem, the tissue being blackened and of a punky texture.

Affected plants do not die quickly but linger on and yield increasingly poor and few blossoms. It is practically impossible to force such plants to increased activity by the heavy application of fertilizers. The foliage of plants affected with this disease is frequently of a lighter green color than that of healthy plants. Probably the number of plants actually killed within the duration of time they are usually kept by growers is very small, but the normal activities of the plant are so materially interfered with that diseased plants can be grown only at a financial loss.

ETIOLOGY

The crown canker disease of rose is caused by the fungus *Cylindrocladium scoparium* Morgan. This organism has been hitherto described only as a saprophyte and, so far as the writer is aware, never reported on the rose.

In 1892 Morgan[1] reported on a fungus collected in Ohio growing on an old pod of *Gleditschia triacanthos* L. The fungus belonged to the group Didymosporæ in Saccardo's[2] system (and to the group Mucedinaceæ-

[1] Morgan, A. P. Two new genera of hyphomycetes. Bot. Gaz. **17**: 190–192. figs. 2. 1892.

[2] Saccardo, P. A. Didymosporæ. *In* Sylloge fungorum **4**: 176–187. 1886.

Hyalodidymæ in Lindau's[3] system subsequently published), but differed from other members of the group in the possession of cylindric spores. Consequently Morgan erected the genus *Cylindrocladium*[4] on the basis of the cylindric character of the spores and used the specific name *scoparium* for the single representative of the genus, probably because of the broom-like nature of the fruiting-bodies.

In July 1899, L. W. Nuttall sent specimens of a fungus on dead leaves of *Asimina triloba* from Nuttallburg, West Virginia to Ellis who in 1900[5] named it *Diplocladium cylindrosporum* E. & E. The specific name used would indicate that Ellis was impressed by the form of the spores. However, the fungus was placed in the genus Diplocladium in spite of the fact that the other members of this group have oval to oblong spores. No reference was made to Morgan's Cylindrocladium.

An examination made by the writer of type material of *Cylindrocladium scoparium* and of *Diplocladium cylindrosporum* shows these two forms to be identical. The descriptions of the two forms vary but slightly in details and comparative studies show sufficient variations to include all differences.

The genus Diplocladium was established by Bonorden[6] in 1851 while the genus Cylindrocladium was not established by Morgan until 1892. The species of Diplocladium described prior to 1892[7] possess more or less oval to oblong spores. There may be some question as to whether or not Morgan was justified in establishing a new genus on the basis of size and shape of spores. The morphological differences in these groups are not very great and the classification at present is very artificial at best. At least a definite morphological basis for separation was utilized so that the writer feels that Morgan was justified in establishing the genus *Cylindrocladium* on the basis of the single species *scoparium*.

As stated by Morgan the spores of *Cylindrocladium scoparium* are 40 to 50 μ long and taper from 4 μ in diameter at the apex to 3 μ at the base. Ellis and Everhart state the measurements of the spores of *Diplocladium cylindrosporum* as being 40 to 50 μ long by 4 to 5 μ in diameter, but mention no tapering. An examination of type material of these two forms shows the spores to be from 36 to 55 μ by 3.3 to 4.51 μ in diameter, the average being about 48.3 by 4.13 μ. They appear to taper slightly but

[3] Lindau, G. Mucedinaceæ-Hyalodidymæ. *In* Die naturlichen Pflanzenfamilien [Engler and Prantl] 1: 1**: 444 447. 1900.

[4] Ellis, J. B. and Everhart, B. M. New species of fungi from various localities with notes on some published species. Bul. Torr. Bot. Club. 27: 49-64. 1900.

Diplocladium cylindrosporum E. and E., p. 58.

[6] Bonorden, H. F. Handbuch der allgemeinen mykologie, p. 98. 1851.

[7] *D. gregarium* Annal. Mycol. 1: 127. 1903 was described by Bresadola in 1903 as having spores measuring 16-27 x 3.5-4 μ.

FIG. 1. CROWN CANKER LESIONS ON ROSE PLANTS

A, stem of an Ophelia plant artificially inoculated with mycelium of fungus; *B*, Hoosier Beauty plant showing cracking at crown; *C*, American Beauty plant showing black water-soaked area at crown. *A* and *B*, natural size; *C*, three-fourths natural size.

it is difficult to detect a measurable difference between the diameter of the apex and of the base of the spores. Spores produced by the fungus causing the crown canker disease of rose are identical in all respects with those of type material of *Cylindrocladium scoparium* and *Diplocladium cylindrosporum*.

The fertile hyphae as stated by Morgan for *Cylindrocladium scoparium* measure 125 to 150 by 5 to 7 μ at the base, while those of *Diplocladium*

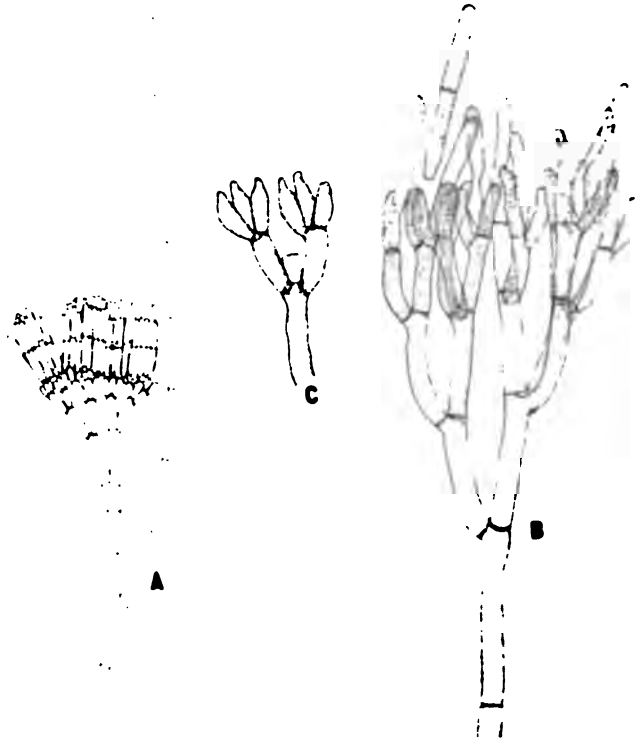

FIG. 2. FERTILE HYPHAE OF CYLINDROCLADIUM SCOPARIUM

A, showing spores held together by sticky substance forming a cylindrical head and fertile hypha with swollen tip; *B*, conidiophore much enlarged showing manner of branching; *C*, terminal branches of conidiophore. *A*, habit sketch. × 233 *B* and *C*, camera lucida drawings. × 700.

cylindrosporum as measured by Ellis and Everhart are 50 to 110 by 5 to 6 μ. The writer in examining type material of the supposedly two different forms found exceptions to both measurements, the range being from 50 to 165 by 5 to 7 μ. Fertile hyphae developed on diseased rose plants placed in a moist chamber and others produced in culture had an

even greater range in length and diameter, so that it is probable that conditions of temperature and moisture under which they develop materially influence their size.

Fruiting bodies of the fungus frequently develop in from two to five days on diseased rose plants when kept in a moist chamber. They are usually borne in fascicles, those observed containing some twelve or more components. The fertile hyphae are erect, hyaline, septate and di- or trichotomously branched (fig. 2). The terminal branches exist in pairs or threes and are arranged in a level-topped cyme of branches. The spores are cut off from their conidiophores by constriction. They are held together by a sticky substance, forming a more or less cylindrical head, but

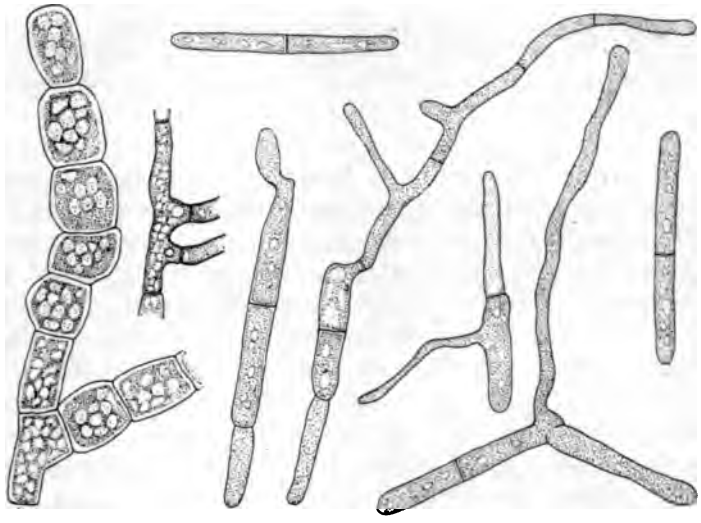

FIG. 3. MYCELIUM AND SPORES OF CYLINDROCLADIUM SCOPARIUM
Some of the spores have germinated. Camera lucida drawings. × 700

separate quickly when placed in water (fig. 2, *A*). Neither Morgan nor Ellis and Everhart mention this point, but an examination of type material shows this character to be present.

Ellis and Everhart[7] note that "with the fertile hyphae are a few much larger simple straight ones swollen at the tip." This character can also be found in type material of *Cylindrocladium scoparium* and the fungus developing in culture from tissue plantings and on diseased rose plants kept in a moist chamber. These sterile hyphae (fig. 2, *A*) may arise from any part of the fertile hyphae but usually exist as the terminal part

[7] Bul. Torr. Bot. Club **27**: 49–64. 1900.

of a main hypha from which the fertile hyphae arise as lateral branches. When the fungus is subjected to extremely moist conditions the swollen tips may resume growth by sending out slender hyphae.

The spores (fig. 3) of *Cylindrocladium scoparium* are solitary, cylindric, 1-septate, hyaline, obtuse at each end and appear to taper slightly from the apex to the base. They are 36 to 55 μ in length by 3.3 to 4.51 μ in diameter, the average being 48.3 by 4.13 μ. Those produced in culture have about the same range. Spores from culture when placed in water on slides germinate after three to twelve hours. Germ tubes may develop from one or both cells (fig. 3).

PATHOGENICITY

On July 21, 1917, twenty-four rose plants, variety Ophelia, growing in the greenhouse were inoculated with cultures of *Cylindrocladium scoparium* growing on potato agar. The fungus had been previously isolated from a diseased American Beauty plant. The plants inoculated were about six months old having been grafted in January on Manetti root stocks. A bit of medium in which the fungus was growing was smeared over the stem of the plants at the graft union, twelve of the plants being previously injured by cutting with a sharp scalpel while care was exercised in handling the remaining twelve to see that they were uninjured. Six plants were similarly treated, except that the medium smeared over the plant contained none of the fungus, three of these plants being injured and three being uninjured. In some cases moist cotton was then wrapped about the inoculated areas while of other plants the infection courts were surrounded with moist soil. The plants were kept moist for the following four days to prevent the inocula from drying.

When examined on July 25 all of the inoculated plants showed typical black, irregular, water-soaked areas at the point of inoculation. The check plants remained healthy. Ten days later there were cracks in the lesions on the inoculated plants which extended in to the wood. Plants in which the infected areas were kept moist with wet cotton showed more pronounced cracking of the bark than those kept moist with soil. In some cases the bark was badly rotted and sloughed away. To date (November 10) the plants have shown no general effect of the disease as compared with healthy plants as check. Tissue plantings were made from five of the inoculated plants and the same fungus obtained in culture.

On August 10, 1917, ten Manetti plants such as are used for stock for grafts were inoculated with cultures of *Cylindrocladium scoparium*. The plants were obtained from Holland during the autumn of 1916 and were growing in the greenhouse in six inch pots. A bit of medium containing

the fungus was smeared over the uninjured surfaces of these plants at the surface of the soil and the inoculated areas were then surrounded with moist cotton. Four plants were similarly treated with the exception that they were not inoculated with the fungus. The cotton on the plants was kept moist for five days to prevent the inocula from drying.

When the plants were examined on August 25 the outer bark had cracked and the tissue about the inoculated areas was somewhat brown and punky in texture. No definite lesions were evident. *Cylindrocladium scoparium* was obtained in culture by means of tissue plantings from inoculated areas. Judging from results of these inoculations it would seem that the Manetti plants were more resistant to the attacks of the fungus than Ophelia plants.

Infection of stems of eight-months-old Ophelia plants was readily obtained at distances of several feet above the soil. A bit of medium containing mycelium of *Cylindrocladium scoparium* was smeared over the surface of the stems, the inoculum being kept from drying by means of moist cotton wrapped about it. Black, irregular water-soaked lesions on the plants were evident after three to eight days. After the removal of the moist cotton the lesions slowly increased in size. No general effect such as wilting or yellowing of parts above the infected areas were evident on September 18 from inoculations made on August 27, although some of the lesions had completely encircled the stem and were an inch or more in width.

Viable spores when sprayed upon plants under favorable conditions also produce infection, as is shown by the following experiment. On August 27, 1917, the stems of six eight-months-old Ophelia plants were sprayed both at the surface of, and eighteen inches above, the soil with water containing in suspension, viable spores from a culture of the fungus. Three plants for a check were similarly sprayed with water containing no spores. The plants were then placed in a large, moist propagating chamber for ninety-six hours. An examination of these plants eight days later showed infection to have resulted. Numerous small, brown areas surrounded by a narrow zone of black water-soaked tissue were apparent on all plants inoculated. The lesions measured from two to six millimeters in diameter. Fourteen days later the lesions had increased very little in size. The fungus was again obtained in culture from plantings from diseased tissue. The check plants remained healthy.

CULTURAL CHARACTERS OF THE FUNGUS

Pure cultures of *Cylindrocladium scoparium* are obtained readily from tissue plantings and from spore dilutions by ordinary methods. Good

vegetative growth and an abundance of spores are produced by the fungus when growing on nutrient agar and agars containing decoctions of rose, prunes, beans, oats and potatoes. The mycelium (fig. 3) is at first white and mostly aerial. After from three to five days it changes to a brownish color, becomes comparatively more resupinate and the medium is changed to a darker color. Medium to which no acid is added is changed to a brown color more quickly than an acid medium.[8] The color imparted to the medium quickly deepens to a reddish brown in from seven to ten days, the ultimate color produced being a mahogany-red. The mycelium changes to a brown color more slowly than the surrounding medium, the aerial mycelium frequently remaining white for months. Usually the mycelium is loose and somewhat tufted. Colonies in petri dishes on an acid[8] agar containing a decoction of potatoes measured four to five centimeters in diameter after five days, and six to eight millimeters after ten days.

Three-weeks-old cultures of *Cylindrocladium scoparium* in petri dishes frequently develop a darker-colored ring consisting of heavy-walled hyphae. Other cultures develop these darker areas more or less irregularly over the entire area of growth after two to three weeks. The cells of these hyphae thicken, assuming a globose form (fig. 3). Well-defined globular bodies which appear to be oil drops soon appear within the cells.

Spores in culture are usually formed abundantly in from seven to fourteen days, their presence often giving the culture a white, granular appearance. They are formed more quickly and abundantly when the fungus is growing on an acid medium.[8] Like those produced on diseased plants subjected to moist conditions, the spores in culture are held together by a sticky substance which is quickly dissolved in water. The spore-masses produced in culture have more irregular outlines than those produced on the host where the tendency is for them to be grouped into a cylindrical head.

Fertile hyphae developed in culture are variable in size, measuring from 100 to 400 μ high by 4 to 9.2 μ in diameter at the base, the average being about 200 by 6.5 μ. The sterile swollen tips are very abundant in culture.

MOISTURE RELATION

Moisture apparently plays an important rôle in the severity of this disease. Lesions on stems well above the surface of the soil resulting from artificial inoculations appear to dry and make no further progress unless kept moist by being surrounded with wet cotton or some such sub-

[8] Three drops of fifty per cent lactic acid were added to each fifteen cubic centimeters of medium, the degree of acidity not being determined.

stance. Inoculations made at a point several inches above the soil frequently result as above. One grower who has had considerable experience with crown canker is of the opinion that the disease is lessened by placing plants with the graft union above the soil, thereby preventing infection at this point. The same grower stated that the seriousness of the disease is reduced by pulling the soil away from the crown of the plant, thus creating a more dry condition at this point. These are undesirable methods, for grafted plants usually develop roots at the graft union when planted sufficiently deep. It is the opinion of the writer that *Cylindrocladium scoparium* is a fungus low in parasitism and that conditions of moisture are important factors in its development.

CONTROL[1]

Although experiments are under way in the hope of developing some method of controlling the crown canker of rose no results are yet at hand. From the nature of the fungus it would seem that control will resolve itself into some method of soil treatment. The fungus grows well on both acid and alkalin media so that the possibilities of control by developing an acid or alkalin condition of the soil do not appear to be promising. Soil sterilization and the exercise of care in using only healthy stock and scions for grafting may be the only feasible method of controlling this disease.

CORNELL UNIVERSITY
ITHACA, NEW YORK

[1] Investigations of control measures are being conducted in cooperation with the American Rose Society and with Professors A. V. Osmun and P. J. Anderson of the Massachusetts Agricultural Experiment Station.

AN EPIPHYTOTIC OF CANE DISEASE IN PORTO RICO

JOHN A. STEVENSON

WITH TWO FIGURES IN THE TEXT

During the past two seasons a most alarming epiphytotic of cane has appeared in Porto Rico, and is still spreading at a rapid rate. This trouble first appeared in the western end of the Island, in the region between Arecibo and Aguadilla, and in the summer of 1916 was practically confined to that area. Since that time not only have its ravages continued in the original territory, but its boundaries have been extended so as to include an area extending from near Bayamón to Añasco, or approximately a quarter of the Island.

It has not been possible as yet to ascertain how long the disease had been present before the first report was received but certainly a year, so that as near as is now known it has been active about three years.

The trouble is as yet confined to the upper reaches of the river valleys, to small enclosed inland valleys, and particularly to fields among the foothills. The broad stretches of the coastal plain, but little above sea level, are still free of disease, although they are planted to the susceptible varieties of cane, and form great continuous areas. The greatest damage has occurred in the rolling stretch of country between Arecibo and Aguadilla, a region which suffers much from drouth. Cane culture has been abandoned on hundreds of acres.

All observations show an apparent tendency of the disease to occur in upland fields. In its eastward progress it has apparently jumped from valley to valley, some distance back from the ocean, or has appeared sporadically rather than working along through the continuous coastal fields.

LOSSES INCURRED

The disease appears to follow a regular course, a given field showing the first year a varying number of infected stools, often only a few, scattered irregularly. There is no apparent loss in this crop. The second year (first ratoon) the greater portion of the field is affected and there may be a loss of from ten to fifty per cent in yield. The third year the growth of

the cane is so poor, and such stalks as are produced are so small, and pithy, that the crop is a total loss.

The loss occasioned is very difficult to estimate since the many fields will show a variation of from one to a hundred per cent of infection and each infected stool, depending upon its age gives a greater or less amount of merchantable cane.

In addition to the decreased yields, there is the loss of all cane which shows evidences of disease, even though it contains a considerable amount of sugar, since the Centrals all refuse to receive cane of this nature, for reasons to be explained later. The figures for 1917 are not yet at hand for comparison, so that it is impossible to give a statement of the monetary loss, but that it already runs into the hundreds of thousands of dollars is agreed. As a single instance, one Central, in spite of three thousand additional acres planted over last year, and the purchase of some thousands of tons of cane from outside districts, dropped behind their previous year's production over half a million pounds of sugar. A considerable number of small planters have been completely forced out of cane growing in two years' time.

NAME OF THE DISEASE

Various names have been used for this disease. It is universally known among the planters as *La Enfermedad*, the disease, and the writer has called it at various times the new disease, the mottling disease, and cane-canker, and at the present moment at least, prefers the use of cane-mottling or the mottling disease. Chlorosis is already in use for a very distinct non-parasitic disease occurring to a limited extent on the south coast of the Island, and hence cannot be used, as has been suggested for this other quite distinct malady.

SYMPTOMS

The one marked and constant symptom of this disease, and the one by which it is easily recognized by any one who has occasion to visit diseased fields, is the peculiar mottling of the leaves. In contrast to the uniform yellowing or whitening of the leaves characteristic of chlorosis, there occur innumerable white or at times yellowish spots and stripes with irregular, indefinite margins (fig. 1). In mild cases the background may be practically normal green, but more often and especially after the first year the leaves are yellow-green to yellow interspersed with the white markings. It is not apparent that mottled leaves die any sooner or are more subject to parasitic leaf fungi than normal leaves.

For some time there are no further symptoms than the mottling, it being impossible to distinguish, except for this one point, diseased from

normal stools. Mottled leaves do not die and fall any sooner than normal ones nor do they cling abnormally to the stalk. The stalks are not stunted or visibly changed internally. It is quite possible to find stalks which show from one or two to ten or twelve lower leaves apparently normal, with all those above mottled. The mottling is apparent as soon as the leaves unfold. The reverse condition of normal leaves above, has not been observed. It is not certain yet whether a leaf which unfolds normally may become mottled later. A varying number of stalks in a stool may show mottling, often only one, more often three or four out of a dozen.

The statements in the previous paragraph represent conditions the first year of infection. The ratoon shoots from all infected stools and from a varying proportion of those that were apparently normal, show mottling from the instant the new shoots are observable. This crop rarely reaches normal conditions of height and stand, more often only thirty to fifty per cent. At this stage, in addition to the mottling, another marked characteristic appears a cankering of the stalks. These lesions appear first, as far as observations show, before the leaf sheaths fall, but after they have become somewhat loosened. At first they are linear spots, somewhat sunken, and brown in color. They soon become ashen or dull gray, and often coalesce to form continuous patches practically covering the internodes (fig. 2). They never, however, pass from one internode to another. They are superficial only, never penetrating for more than 1 to 2 mm., except along such cracks as occur. Even here the reddening that is found is seldom more than that usually found in such locations in normal cane.

The cracking or splitting is not considered a symptom, being merely a result of the drying of the cane. Splitting normally occurs in many varieties, although of course it is more marked with this disease. There is no internal red rot or other form of rot accompanying the cankers, but there is a shrinking of the internodes and a general condition of pithiness and lack of juice. All cankered canes show mottling of the leaves, but the reverse is not true.

It may be noted at this point that not only is there a lack of juice in cankered canes but what does occur is of an objectionable nature. A very high glucose ratio is reported (non-crystallizing sugars) and the juice behaves badly during clarification and other processes to which it is subjected. A comprehensive series of chemical tests is about to be made at this Station, to be reported upon later.

CAUSE

Continued efforts have been made to ascertain the cause of the mottling disease but it still remains obscure. During the first year, as will be noted in the first report on the disease,[1] the opinion was held that the condition was merely the result of too extensive planting, without seed selection or proper cultivation practices acting in combination with very unfavorable weather conditions—a drouth followed by an excessive wet period, resulting in what is commonly referred to in the literature of cane diseases as an epiphytotic of root disease. It has become only too apparent, however, since then that there is more involved than merely a situation arising from bad weather and bad agriculture.

FIG. 1. HEALTHY AND MOTTLED CANE LEAVES

The usual course of procedure to find a parasitic cause has been followed; various fungi have been investigated, cultured, and used for inoculation; and all without result. The juice of mottled stalks has not proved infectious. However, it has been found that "seed pieces" taken from stalks showing mottling, invariably produce mottled shoots no matter how carefully they may have been disinfected. The "seed pieces" used appeared to be normal both externally and internally. The leaves

[1] Stevenson, John A. Cane disease. Rept. Board of Comm. of Agr. Porto Rico 5: 58-71. 1917.

were removed to make sure and in some cases the pieces were planted in sterilized soil. Such further tests as have been made with soil of diseased fields and so forth, have been inconclusive.

The broad question of degeneration of varieties comes up at this point, and is considered the cause by many. If the white cane only were involved, a type which has been grown here for many years, it might be an

FIG. 2. HEALTHY AND DISEASED STALKS OF CANE

easy way out of a very difficult situation to lay the entire blame on this general term but other, and some of them comparatively new varieties are attacked as well. However, it is not impossible that degeneration is involved to some extent.

VARIETIES AFFECTED

Most of the cane of the affected district has been of two varieties: the striped or *rayada* and the white (*blanca*) or *Otaheite*, probably the same as the old Bourbon cane. The white cane was first affected and is at present

most subject to the disease, the cankers being especially characteristic of this variety. Its elimination, as has already occurred in other parts of the Island, seems certain. In many places the *rayada*, during the present year, has been as badly affected as the white, although there is still the possibility that strains from outside districts may remain immune.

Other varieties grown on a smaller scale and brought in for trial have been uniformly affected, *bamboo*, *penang*, *B-3412*, *B-208*, *yellow caledonia*, *Cavengerie* and others. A dark red variety, locally known as *sarangola* has been fairly resistant but unfortunately is not a good milling cane, nor is it probable that it would have any great degree of resistance if planted on a large scale. New seedling varieties are to be tried as rapidly and extensively as possible.

COMPARISON WITH OTHER CANE DISEASES

In order to distinguish the mottling disease from the other common and at times serious cane diseases which might be confused with it, or which are to some extent connected with it, some account of these will not be out of place here.

The more the disease is studied the more it appears to resemble the mysterious *sereh* of Java. It is unlikely that it is that exact disease, but it is not improbable that it is of the same general nature, produced by the same or similar environmental factors. Many of the symptoms are the same, although none, it must be admitted, are those that are considered essential. Some of the important points of similarity are: the course of the disease over three years, the stunting of stools and shortening of internodes (in advanced cases), the fact that the disease is carried over from old plants to new ones by cuttings, and a poor development of the root system. On the other hand, this new disease in addition to the leaf mottling and stalk cankers not ascribed to sereh, does not show gumming, internal red lines, more disease at the base of the stalk, or the abnormal stooling giving the grassy appearance from which the sereh takes its name.

Rind disease caused by *Melanconium Sacchari* Mass. as a factor was quickly eliminated, no more of this fungus being present, than can be found in normal cane. In the very heart of the affected territory a large field of *rayada* cane was found which had been allowed to become over-mature and was rapidly going to pieces under this fungus, but there was absolutely not a sign of mottling.

Other stalk diseases were as easily removed from consideration by the absence of their respective symptoms or the fruiting bodies of the causative fungi. There was little, if any, internal red rot (*Colletotrichum falcatum*), brown rot (*Diplodia cacaoicola*), dry rot, (*Nectria* sp. and other

forms) or of *Cytospora Sacchari*, serious at times as a cause of disease. These as well as other fungi, (*Trichoderma lignorum*, *Fusarium* sp., *Valsa* sp., *Leptosphaeria Sacchari*, and others) were common at times, but only as saprophytes as was demonstrated by inoculation tests.

It is not so easy to ascertain the relation of what is commonly known as root disease, and certain phases of the problem are yet unsolved. It is the common assumption of most writers on cane disease that certain fungi, notably *Marasmius Sacchari*, when favored by weather and cultural conditions, act parasitically on the roots of the cane, producing stunting and final death of the stools. It is, however, not at all clear which of the various fungi found in such locations are truly parasitic, if any, and which only saprophytic, and, above all, whether the weather and cultural conditions above mentioned are not in reality primary, making it possible to relegate the fungi to a secondary status.

In the affected territory under consideration, a region habitually subject to drouths, and where good agriculture is the exception and not the rule, the presence of root disease, especially on the long-time ratoons attempted, becomes almost universal, so much so that for a long time in agreement with all agriculturists and others who investigated the situation, the writer considered the trouble root disease. However, more extensive studies have necessitated a decided change of opinion. Fields have been seen on land never before in cane, which showed mottling but no root disease and on the other hand fields have been encountered in the last stages of root disease but without a sign of mottling. It now appears beyond any reasonable doubt that there are two diseases to be considered. One a disease characterized by a mottling of the leaves and later by a cankering of the stalks, and the other the old so-called root disease marked by rotten roots permeated by a white mycelium, and by a binding together of the lower leaf sheaths in characteristic fashion. Both may be present together with particularly disastrous results for the cane, or either may be present alone, with a similar result as far as the cane is concerned.

CONTROL

Practically every conceivable measure which has ever been recommended for the control of cane disease and especially those usually given as efficacious for root disease have been tried, and without any other result than the continued progress of the disease. Liming, increased cultivation, treatment with bordeaux mixture, seed of established varieties brought from outside regions, seed of new varieties, and the use of land not before planted to cane, have been tried.

It is apparent that very drastic measures will be necessary to check the

epiphytotic. The foremost requirement apparently will be the introduction of a rotation system, (a heretofore unknown practice in Porto Rican cane culture), and one which will include a legume. In the meantime continued efforts are being made with new seedling varieties, particularly those produced at this Station, and it is hoped that some will ultimately be found which, under proper care, will succeed.

In many instances the growers are turning to other crops or pursuits—tobacco, cotton, cattle, and to some extent minor food crops. There is no reason why it would not be possible to combine one or more of these in a rotation with cane so as to maintain the latter industry. Whatever the outcome it will at least result in an absolute revolution in the cane agriculture of the Island.

SUMMARY

A new and alarming cane disease has appeared in the western end of Porto Rico and is spreading rapidly. The loss which varies greatly from field to field, being complete in the third year of the disease, has already reached a total of hundreds of thousands of dollars.

The disease is known as the mottling disease of cane.

It is characterized by a peculiar white mottling of the leaves and in advanced stages by gray cankers of the rind of the stalks and by a shrinking and drying of the stalks proper.

The cause is obscure, although possibly due in part to weather conditions and poor agricultural practices. Of the many fungi found in and about cane fields none are considered to have any direct connection.

All varieties are affected, especially the native white or *Otaheite* and the *rayada*.

The mottling disease has some characteristics in common with sereh, but there are also many points on which the two diseases differ. Rind disease and other diseases of the stalk have no causative connection with mottling. Root disease is also distinct, but may occur in connection with it.

No definite control is known.

INSULAR EXPERIMENT STATION
DEPARTMENT OF AGRICULTURE AND LABOR
RIO PIEDRAS, PORTO RICO

THE EFFECT OF ROENTGEN AND ULTRAVIOLET RAYS UPON FUNGI

H. L. Trumbull and J. W. Hotson

With Two Figures in the Text

The museum of the University of Washington, Seattle, which served as a forestry building (fig. 1) during the Alaska-Yukon-Pacific Exposition in 1909, was built of logs of green Douglas fir (*Pseudotsuga taxifolia*) and Western hemlock (*Tsuga heterophylla*). Five years after the building was erected, it was observed that many of the logs showed signs of decay resulting from a serious attack of a wood-destroying fungus, *Fomes pinicola* (Fr.) Cke. Each succeeding year an increasing number of sporophores appeared over the logs varying from the white rounded buttons (fig. 2, *A*) just protruding from the bark to well developed shelving forms. Where deep notches were cut into the logs, the characteristic red rot in the heartwood with the broad, white, felted masses of mycelium was readily detected. Occasionally also the sporophores of *Polyporus schweinitzii* Fr. (fig. 2, *B*) appeared on these logs, but by far the greater damage was done by *Fomes pinicola*. It was with the latter that the following experiments were made.

Upon recommendation of the forestry department, means were devised to combat the ravages of these fungi, including the installation of a heating system to facilitate the drying of the timbers and impregnation of the wood with fungicides, notably copper sulfate and mercuric chlorid. Although the experiments with copper sulfate were the most successful, the impregnation was observed to proceed only in a longitudinal direction and for short distances. This was doubtless due to the high water content of the cells and to the size of the logs which are five to six feet in diameter and varying from forty-two to fifty-six feet in length.

On account of the partial success of the trials with copper sulfate further experimental work with it was contemplated. It was the intention to apply a considerable quantity of a solution of this compound to the top of each of the vertical log columns and allow it to percolate downward. In order to do this it would have involved raising the roof of the building, putting a tight fitting collar around the top of each log and filling this space with the fungicide. Although this was the most promising mode of controlling the disease, the cost of such an experiment precluded it.

FIG. 1. FORESTRY BUILDING, UNIVERSITY OF WASHINGTON

The immense logs are attacked by Fomes pinicola and Polyporus schweinitzii.

FIG. 2. SPOROPHORES OF FOMES PINICOLA AND POLYPORUS SCHWEINITZII

A, Fomes pinicola, showing a white rounded button and a fairly well developed bracket; *B*, Polyporus schweinitzii.

It was in the hope that a feasible remedy might be found that the following experiments were undertaken.

From the large mass of data[1] relative to the action of X-rays on living organisms, it seemed reasonable to suppose that the spread of the fungus might be checked by this agency. From the work of numerous investigators it has been shown that X-rays together with rays from radioactive sources exert a profound influence upon organisms, in some cases, modifying and, in others, totally inhibiting life processes. Thus, in summarizing the work in this field, Gager[2] states that Roentgen and radioactive rays produce similar physiological effects and that sensitiveness to these rays varies with the species in the case of plants as well as animals. He further states that the sensitiveness varies with age, the older tissues being less sensitive. Bardeen's experiments[3] with the ova of toads and frogs show that exposure to X-rays results in abnormalities in the embryos.

It should be stated, however, that there are cases on record[4] in which exposure to X-rays has failed to produce a noticeable effect on the life processes of organisms, particularly in the case of bacteria. Weinzirl,[5] in an investigation along this line, found that cultures of *Bacillus coli* and of *Bacillus tuberculosis* were not affected in any way by exposures varying from five to thirty minutes. The cultures employed by him were one day and thirty days old respectively.

[1] Richards, A. Recent studies on the biological effects of radio-activity. Science n. s. **42**: 287. 1915. The effects of X-rays on the action of certain enzymes. Amer. Jour. Physiol. **35**: 224. 1914. Experiments on X-radiation as the cause of permeability changes. Amer. Jour. Physiol. **36**: 400. 1915. The effect of X-rays on the rate of cell division in the early cleavage of Planorbis. Biol. Bull. **27**: 67. 1914.

Bardeen, C. R. Abnormal development of toad ova fertilized by spermatozoa exposed to Roentgen rays. Jour. Exp. Zool. **4**: 1. 1907. Variations in susceptibility of amphibian ova to the X-rays at different stages of development. Anat. Record **3**: 163. 1909. Further studies on the variation in susceptibility of amphibian ova to X-rays at different stages of development. Amer. Jour. Anat. **11**: 419. 1911.

Packard, Charles. The effect of radium radiations on the fertilization of Nereis. Jour. Exp. Zoöl. **16**: 85. 1914.

[2] Gager, Charles Stuart. Effects of the rays of radium on plants. Mem. New York Bot. Garden **4**: 1-278. 1908.

[3] L. c.

[4] Russ, Viktor K. Einiges über den Einfluss der Röntgenstrahlen auf Mikroorganismen. Archiv f. Hygiene **56**: 341-360. 1906.

Allen, Charles W. Radiotherapy and Phototherapy. p. 363. 1904.

[5] Weinzirl, John. Private communication to the authors, based on unpublished data.

EXPERIMENTAL

Samples of the infected wood and of the sporophore of the fungus (*Fomes pinicola*) were collected from a number of logs and subjected to varying exposures of X-rays. These samples were kept in sterilized bottles before and after exposures. The media used for cultures were potato- and prune-agar, using twenty grams of agar to the litre. The latter medium proved the more favorable for the growth of the fungus. The samples for cultural study were in each case taken as far as possible from the center of the specimen, which was removed from the container, cut with a sterilized knife or broken, and a small portion taken from a location that seemed least affected by the rays. The mode of recognizing the mycelium of *Fomes pinicola* in culture tubes was more or less imperfect. Known pure cultures of the fungus were grown on the same media as those of the experiments. These were used for comparison as to the gross and microscopic appearance of the mycelium. As a species of Penicillium was the only other form appearing in these cultures, it did not prove a difficult task to distinguish them. Apparently the X-rays had no inhibiting effect on the growth of the Penicillium. For comparison three tube cultures were made of each sample before exposure and the same number after. The growth of the cultures was observed and compared at intervals of four to ten days over a period of several months.

Experiment 1. Four samples of wood from infected logs were exposed to the rays from an ordinary Roentgen tube for over an hour at a distance of about eight inches from the bulb and directly opposite the anticathode, at a potential which was not known accurately, but which corresponded to a spark gap of about four to six inches, the humidity of the air being high. The cultures from three of the exposed samples showed healthy growth at the end of a week and exhibited no exceptional features by comparison with the control cultures. The fourth culture as well as one of the controls failed to grow.

It seemed advisable to carry out further experiments so that the samples could be subjected to repeated and more intense exposures, such for example, as those furnished by the Coolidge tube. Through the kindness of Dr. J. H. Snively of Seattle, the authors were permitted to make use of one of these tubes in performing the experiments which are described in the following paragraphs. The exposures were made under conditions substantially like those employed so widely at present by practitioners of radiotherapy. The main advantage of the Coolidge tube over those of the older type for work of this sort is that reproducible conditions can be realized by controlling the voltage and amperage of the thermionic current.

Experiment 2 (with a Coolidge tube). Two samples of infected wood and ten samples of the sporophore of the fungus were exposed for inter-

vals of six to twenty-five minutes on December 31, January 5, and January 20, thus effecting a total exposure of from seventeen to sixty-five minutes. The samples were kept in sterilized bottles after each exposure, the cultures being made within a day after the final one. The exposures on the first two dates were made with an eight and a half inch spark, and eight milliamperes, the one on January 20 being made with a six-inch spark and eleven milliamperes. Those samples which were longest exposed to the rays from the Coolidge tube were subjected to about fifty times the so-called "erythema dose," (a dose employed in X-ray therapy sufficient to produce a distinct reddening of the skin). The results of the above tests showed that in none of the cases was the normal character or rate of growth interfered with by exposure to X-rays even though the treatment was repeated and the rays were of great intensity.

Experiment 3. On April 9 tube cultures taken from the samples exposed on January 20, referred to in experiment 2, were treated for eight minutes with rays from a Coolidge tube using a current of ten milliamperes and a spark gap of eight and one-half inches. On the following day transfers were made from these treated cultures using the superficial mycelium and also gouging out some of the agar containing hyphae. Cultures from this superficial mycelium failed to grow, but in every case where samples were taken by gouging out large pieces of agar, normal growth was produced.

Experiment 4. The rays (ultraviolet) from a quartz mercury vapor lamp, operating at 110 volts and 57 ohms were directed against thin sections of the sporophore for one hundred minutes. The sections varied in thickness from one to ten millimeters. Cultures made from the thinnest layers failed to produce the mycelium of *Fomes pinicola*, but this was probably due to a drying process rather than the result of the X-rays. Cultures taken from the interior of samples eight to ten millimeters in diameter produced a normal growth.

As the object of this investigation was to discover a remedy that would apply to a particular case and as the necessary equipment to undertake a more extended study involving other fungi was not available, the authors have discontinued further work along this line, at least for the present.

CONCLUSIONS

It is obvious that from these limited data one is not justified in drawing any general conclusions. It would seem, however, that the method employed in the preceding experiments is not suitable for the control of such wood-destroying fungi as *Fomes pinicola*. In general, cultures taken from the external portions of exposed samples usually failed to grow. This

also frequently occurred with the controls under similar conditions. These results were probably due to partial desiccation, and in so far as the X-rays helped to furnish this condition, it was effective, but samples of mycelium taken a centimeter or more from the surface of treated specimens usually grew.

As the results obtained in these experiments were contrary to expectation, a test was made of the efficiency of the Coolidge tube that was used. The right hind quarter of a guinea-pig was exposed to double the "erythema dose." No noticeable effect was seen the first two months. At the end of three months, however, the hair had all sloughed off over this area and nowhere else. This and other experiments with guinea-pigs showed conclusively that the Coolidge tube that was used was highly satisfactory.

Although the results of these experiments are largely negative they are thought to be of sufficient interest to warrant their publication.

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

SCLEROTINIA TRIFOLIORUM, THE CAUSE OF STEM ROT OF CLOVERS AND ALFALFA

A. H. Gilbert and C. W. Bennett

With Five Figures in the Text

The first recorded occurrence of stem rot of clover, according to Eriksson (1), was in Germany in 1857; and the first case in which this disease, under the name of clover sickness, was attributed to *Sclerotinia Trifoliorum* Erik.[1] as the cause, was, according to the same authority, reported by Hermann Hoffman in 1863.

In England, investigations of clover sickness by Lawes and Gilbert date back to 1849 and were continued through nearly thirty years, but it was not until about 1898, according to Güssow (2), that a fungus was recognized as at least one of the chief causes of the trouble. In 1872 Emil Rehm (3) published a paper containing a full description of the disease, the life history of the causal organism and suggestions as to treatment. Rehm's paper refers to the organism as *Peziza ciborioides* Fries. This name was discarded as untenable by Eriksson (1) who substituted the name *Sclerotinia Trifoliorum* Erik. as more suitable. Dr. C. L. Shear of the Department of Agriculture, Washington, D. C. kindly investigated for the writers the matter of the nomenclature and reports that the name *Peziza ciborioides* Fries as used by Rehm, and also *Sclerotinia ciborioides* as used by H. Hoffman in Icon. Anal. Fun. 1863, were both erroneous in referring to this fungus on clover, the use of these names being due to a misidentification of Fries' species which we have no reason to believe was the same as the fungus under consideration.

Rehm's paper reports the disease as very serious in certain sections of Germany. Attention is called to its economic importance in that it threatened the complete destruction of the clovers upon which great dependence is placed by the farmers for forage and soil restoration. The disease is termed by Rehm clover rot (Kleefaule), and is reported by him upon red clover (*Trifolium pratense* L. and *T. pratense* var. *saturum*), crimson clover (*T. incarnatum* L.), white clover (*T. repens* L.) and bastard clover (*T. hybridum* L.). Rehm reported it as not occurring upon alfalfa or upon any other related leguminous hosts.

[1] The fungus now known as *Sclerotinia Trifoliorum* Erik. was first erroneously referred to *Peziza ciborioides* Fries.

Eriksson (1) reports the occurrence and spread of the disease in Sweden in 1878–79, and also that it was first observed in the province of Hesse in Germany in 1857 where it attained an epiphytotic character. In 1870 it had spread to Denmark. In the same year Kühn (4) also writes of the disease under the name *Peziza ciborioides* Fries. That the disease is still prevalent in some parts of Europe is learned through correspondence from F. K. Ravn of Copenhagen who wrote in 1915 that it was of considerable significance in Denmark. Ravn gives, in addition to the clovers, as a host plant, alfalfa, upon which the disease was prevalent. Neither Rehm nor the other European writers mentioned the occurrence of the stem rot on alfalfa, in fact, Rehm specifically states that alfalfa was not a host. With respect to this, some change has perhaps taken place in the parasitic relations due to changed cultural conditions, by which the disease has become more virulent on alfalfa. At the earlier period it may have occurred slightly but not enough to be noted.

The disease in question has been known by various names. In Europe it has been designated as clover rot (Kleefaule), clover cancer (Kleekrebs), in England, clover sickness, and in America, clover rot, wilt, root rot, and stem rot, of clovers and alfalfa. In the work connected with this paper the name stem rot has been uniformly employed.

The occurrence of stem rot in America seems to have been first recorded in 1890 by Chester of Delaware (5). He reports its occurrence upon crimson clover and cites the earlier work of Rehm and others in Europe. The recent reports from various states, as well as the observations made by the writers in Kentucky, suggest that the disease is becoming more prevalent in this country. As reported previously (6), stem rot of clovers and alfalfa has been recognized in the following states: New York, New Jersey, Virginia, Indiana and Oregon. No doubt it occurs also in other states from which no reports have been received by the writers. So far as we have been able to ascertain, the first report in this country of the occurrence of *Sclerotinia Trifoliorum* as the cause of stem rot of alfalfa was made by the senior writer as a joint author in 1915 (6). The earlier references to stem rot or root rot disease caused by this fungus have been upon clovers and especially upon crimson clover. In 1908, Stewart, French and Wilson (9) reported a wilt of alfalfa which they claimed to be caused by *Sclerotinia libertiana* Fckl. It was, according to the writers, first thought to be *S. Trifoliorum* Erik., but was identified by R. E. Smith as *S. libertiana* Fckl. From the manner of attack described, the present writers are inclined to think that the trouble was not the stem rot under consideration. In the case of the New York disease the points of attack were said to be a foot or more from the root. In all our observations of the stem rot disease the point of attack has been almost without exception

on the stems near the surface of the ground. Again, the New York bulletin states that on certain alfalfa plants near the surface of the ground the fungus produced "a luxuriant cottony growth." We have never observed *S. Trifoliorum* to develop a vegetative growth on the stems which would be called luxuriant. Rather, the early activity of the mycelium is within the stems and when the fungus appears on the surface it is to form the sclerotia and the growth is compact, white and velvety. The writers of the New York bulletin speak also of a crown rot of red clover observed at Phelps, New York, in 1901. From this brief reference the present writers would strongly suspect the red clover trouble there to have been caused by *Sclerotinia Trifoliorum* Erik.

THE DISEASE

The first observed effects of the disease is a wilting of some or all of the leafy portions of the plant. This result may be seen in the fall and early spring. In Kentucky the fungus is active during the winter months of February and March, when it may be found rotting young plants or affecting the tender shoots of older plants. A careful investigation of the wilted plant will generally disclose at the base of the stems and near the surface of the soil a fungous growth consisting of a compact mass of white hyphae. Early stages of the disease show a discoloration of the stem just beneath the crown and extending downward (fig. 1, A). The cushiony masses of white hyphae appearing on the surface of the stems later develop into black sclerotia. These are variable both in shape and size. Some are nearly spherical and as small as a radish seed, while others are irregularly flattened or elongated or spherical and as large as a small pea. The majority of them are of the latter shape and size. They occur commonly on the surfaces of the stems just above the surface of the soil, but are also formed in the centers of stems and sometimes on branches at some distance from the ground. The last condition is much less common. With the maturing of the sclerotia the affected stems are completely decayed, the upper parts of the plant soon fall down and the decay involves also the roots (fig. 1, B). The final result in late spring and summer then is that a cluster of black sclerotia are found in the soil where a plant has been. The disease spreads from centers of infection and the plants are killed in areas of various sizes throughout the field. Alfalfa or clover sown in the fall or spring are attacked the following winter and spring, and in numerous cases observed, from 25 to 50 per cent or more of the stand has been destroyed. Alfalfa may be attacked also during the subsequent years but the damage is not so serious as during the first year.

HOST PLANTS

In Kentucky the stem rot has been observed upon alfalfa, crimson clover, red clover, white clover, and on one occasion, upon a common weed, *Euphorbia maculata* L. Crimson clover is by far the most susceptible of the hosts in Kentucky, alfalfa stands next and the remaining hosts are slightly less susceptible. The host plants as reported by Rehm have

FIG. 1. STEM ROT OF ALFALFA

A, An early stage of the disease appears in the discolored portion of the stem just below the crown; *B*, the roots of these plants have been destroyed by the fungus and sclerotia have formed at the points of decay.

already been noted and these agree with reports from other European writers, with the exception that alfalfa is reported as a host in Denmark by Ravn. Güssow reports red clover as a host in Canada.

LIFE HISTORY OF CAUSAL ORGANISM

The sclerotia which are formed in the spring lie in the ground during the summer, germinating in September and the later fall months to form apothecia (fig. 2). The apothecia are disk shaped, at first concave on their upper surfaces, later becoming flat, and still later convex, when the

margins crack at several points. They are from 3 to 8 mm. in diameter and are borne on stalks from 4 to 25 mm. in length. Apothecia have been found in the field from October to December in Kentucky. After that time very few, if any, may be found. After the first warm, moist days in February, growing hyphae may be found on the surface of the soil in the field and soon after infection of the host plants may be observed. Sclerotia are again formed in from two to several weeks and the life cycle is completed.

FIG. 2. SCLEROTIA AND APOTHECIA OF SCLEROTINIA TRIFOLIORUM

OBSERVATIONS ON THE GENERAL MORPHOLOGY OF THE ORGANISM

Chester (5) suggests that the mycelium of *Sclerotinia Trifoliorum* also produces conidia following the analogy of other members of the genus, but numerous observations made by the writers both in the field and in pure cultures of the fungus have failed to disclose any conidial stage. Minute spore-like bodies or gonidia are formed on the surfaces of ripe apothecia in the field under certain conditions, and also in pure cultures of the organism in test-tubes. A histological study of the apothecia from the field showing this gonidial development resulted in the following observations:

The ascospores from many of the asci had been discharged leaving the asci empty. Ascospores remaining in their asci and also spores lying

upon the upper surface of the apothecium had germinated forming a tufted growth made up of short, club-shaped gonidiophores, and from these numerous small, globose spores had been produced. Entirely similar branches and spore-like bodies have been found on the vegetative mycelium growing in pure culture in a test-tube (fig. 3). These gonidia have in no instance been observed to germinate. It is not clear under

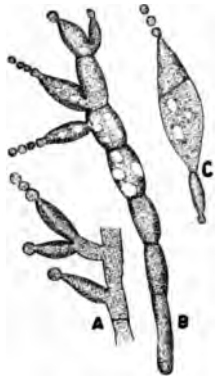

FIG. 3

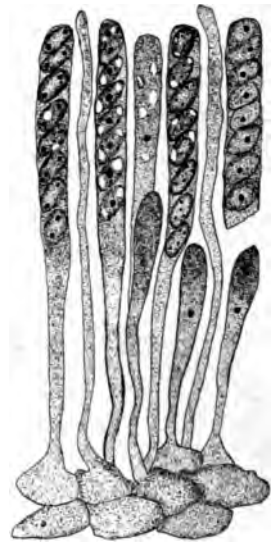

FIG 4

FIG. 3. GONIDIAL FORMATION OF SCLEROTINIA TRIFOLIORUM

A, Gonidia on surface of an apothecium; *B* and *C*, gonidial formation in hanging-drop culture of ascospores. Drawings by A. H. Gilbert.

FIG. 4. ASCI AND PARAPHYSES FROM APOTHECIUM OF SCLEROTINIA TRIFOLIORUM

The group includes young uninucleate asci, and mature 8-spored asci in three conditions as to ascospores, viz., one, two and four nucleate. × 400. Drawing by A. H. Gilbert.

just what conditions in the field ascospores germinate to form the basidium-like stalks and gonidia, rather than to form vegetative hyphae. It may be that those ascospores which are forcibly discharged from the apothecium regularly form vegetative hyphae in the soil, while ascospores remaining in the apothecia germinate instead to form the above described structures.

Several apothecia have been sectioned, having been first killed in Flemming's fluid, and have been stained with safranin, gentian and orange G.

The structure of the hymenium is similar to other Peziza-like forms, being made up of closely set, eight-spored asci, intermingled with slender paraphyses which normally project slightly beyond the tips of the asci. Among the mature asci and projecting only about half way to their tips are numerous young asci containing one nucleus. In a number of fixations and cuttings no traces of the three divisions which result in the final eight nuclei and eight spores were found. This is no doubt due to the fact that the fixations were not sufficiently rapid or were not made at suitable times. It is noted, however, that nuclear division has occurred in the mature ascospores and that in a group of adjoining asci may be found

FIG. 5. CULTURES OF SCLEROTINIA TRIFOLIORUM SHOWING FORMATION OF SCLEROTIA IN CONCENTRIC RINGS

those with one, two and four nuclei. This division of the single nucleus of the mature ascospore is said to be of common occurrence in the ascomycetes (13) (fig. 4).

PARASITISM AND SAPROPHYTISM

Our observations show that the fungus may persist in a field for several years, at least, although less damage is done to the crop after the first year. Recent observations in two fields of alfalfa that are three years old show the disease to be still present, numerous plants being killed during this spring, 1917, and many spots throughout the field, now occupied by weeds, show where the plants have been destroyed in previous years. In numerous instances the young, tender shoots of old plants are affected and killed, and it appears that this beginning may lead to the death of the entire plant, though apparently it takes a longer time to accomplish this than in the case of the young plants.

It is claimed by De Bary (7) that *Sclerotinia ciborioides*, which we assume to be the same as *S. Trifoliorum*, may go through the whole course of its life as a saprophyte. The writers have seen no indications of this in their observations but recognize that it is of course possible. Such an existence would easily explain the sudden appearance of the disease in a field where for years no clover or alfalfa had been grown and where there seems to be no clear source of infection. Several instances of this sort have been noted.

ARTIFICIAL INFECTION

Artificial infection was accomplished in the greenhouse in several ways. Small portions of mycelium were placed in contact with a stem or leaf of young alfalfa and in a few days the leaves began to wilt due to the invasion of the fungus. This method, however, was not successful without covering the plant first with a bell-glass. Bits of agar containing mycelium were placed on the surface of the soil in pots containing young clover plants and a diseased condition of the plants induced in a short time. The spread of the fungus on the seedlings in the greenhouse s easily checked by cutting down the water supply.

ASCOSPORE DISCHARGE

Rehm claims to have shown experimentally that ascospores bring about infection. He hung ripe apothecia over young plants and mycelium was produced within the leaves in six to eight days. This point has not been investigated by the writers but some observations on ascospore discharge have been made.

To determine whether or not the ascospores are forcibly discharged from the asci, mature apothecia were taken from the field and with their sclerotia transferred to moist chambers. Each dish was prepared by placing an agar film on the inner surface of the cover. The apothecia with their sclerotia imbedded in soil were placed on wooden blocks at varying distances from the agar film. The ascospores discharged from the apothecia so placed were caught upon the agar film and their presence indicated by the development in a few days of a hyphal growth. It was found that spores reached the agar surface and took effect at distances of from 10 to 20 mm. and that the diameters of the areas inoculated by these discharges was 7 to 10 mm. At distances of 25 and 30 mm. no inoculation was secured.

THE FUNGUS IN PURE CULTURE

The organism has been grown upon the following agar media: synthetic,[2] cornmeal, oatmeal, potato (hard), alfalfa infusion; and on potato plugs. An abundant development of sclerotia was secured on the synthetic medium and also upon the alfalfa infusion agar. On potato plugs a dense, tufted mycelium and large sclerotia were produced. On potato (hard) and oatmeal agars the vegetative growth was very thin. When the reaction or the food supply of the medium is not favorable very small sclerotia are formed around the edge of the agar surface and on the sides of the tubes or plates.

In tubes and petri dishes the sclerotia are regularly developed in circles around the centers of inoculation (fig. 5). The length of time required for their development varies somewhat with the temperature. At ordinary room temperature a circle of mature sclerotia is developed in about ten days after inoculation. At 5°C. they are formed in from fifteen to seventeen days.

DISTRIBUTION

The question as to the source or cause of the rather widespread infection in Kentucky in 1914 and subsequently has been a problem in the minds of the writers and is still unexplained, at least if we do not accept the theory that the fungus remains saprophytically in the soil. No doubt climatic and crop conditions have had an important bearing on widespread virulence.

The local distribution of the fungus it seems may be explained easily. The spores which are formed in the apothecia in the autumn may be carried from one field to another on cultivating tools or on vehicles passing from field to field, or, the sclerotia which form in considerable abundance in the spring may be taken from the field in cuttings of the crop and then distributed in manure or waste materials to other fields. The sclerotia are apparently not able to germinate until after a period of rest, for there are otherwise favorable conditions for germination, viz., temperature soil and moisture conditions, for some weeks in the spring after the sclerotia are formed and no germination has been observed to occur at that time. It should perhaps be noted in this connection that practically all the activity of this fungus is during the cool weather of late fall, early winter and early spring.

CONTROL

The German and English investigators of this disease have suggested no feasible means of control except the rotation of crops in which none

[2] The formula is that given by Duggar, B. M. Fungous Diseases of Plants, p. 26.

of the host plants is grown for a number of years. Special measures partially effective have been proposed by some. Ritzema Bos, a Dutch writer (8) recommends digging out the plants from infested parts of clover fields and burning them. Rehm recommends abandoning the fields to rotation of crops and also suggests deep plowing to bury the sclerotia too deep for germination. The writers believe that there is considerable efficacy in the latter suggestion, in fact some good results have already been observed from this treatment.

Deep plowing together with liming is then suggested as a control measure with the alternative of rotation of crops, leaving out clover and alfalfa for a period of from three to five years. The efficacy of the latter method depends upon whether or not the fungus lives saprophytically in the soil in the absence of the leguminous host plants, and this point has not yet been settled. Precautionary measures against the transportation of sclerotia and spores on cultivating tools or in other ways should, no doubt, be carefully practiced.

In the spring of 1916 some data were collected in Logan County, Kentucky as to the preceding crops in cases of more or less seriously affected fields of crimson clover and alfalfa, and as to the effect of different rotations upon the amount of stem rot occurring. It was observed that the absence of a leguminous crop, such as clover or alfalfa, immediately preceding the host crop did not preclude the occurrence of a serious attack but that in the majority of cases the trouble was more serious where red clover, crimson clover or alfalfa had been grown for one or more years preceding the crop affected.

SUMMARY

Stem Rot of clover was reported from Germany as early as 1857, and the causal organism determined as *Sclerotinia Trifoliorum* in 1863. A paper by Emil Rehm in 1872 gives a full description of the fungus and the disease induced by it, including some data as to its control. The disease has also been studied in England and reported from Sweden, Denmark and Canada. In the United States it is reported from New York, New Jersey, Virginia, Indiana and Oregon, and probably occurs in other states.

The host plants reported by Rehm are red clover, crimson clover, white clover, and bastard clover. Ravn of Denmark reports it upon alfalfa in 1915.

The first published report of this stem rot upon alfalfa in the United States was by Gilbert and Myer in 1915. The disease had previously been reported upon red clover and crimson clover by Chester in 1890 and by other writers.

The disease causes a wilting of the leaves and stems and a rotting of the stems and root systems, accompanied by the formation of black sclerotia. The sclerotia are formed in Kentucky in February, March and April and germinate in the autumn to form apothecia. Ascospores are discharged in the autumn and early winter and germinate to form hyphae which attack the host plants in early spring.

Laboratory experiments show that the ascospores are forcibly discharged from the apothecia. Agar surfaces were infected at a distance of 20 mm. from the apothecia. No results were secured at distances of 25 mm. and 30 mm.

Greenhouse experiments resulted in producing the disease by inoculating young clover and alfalfa plants with a pure culture of the fungus. In pure culture the organism has been grown on various media. The most abundant vegetative growth occurred on potato plugs and the greatest development of sclerotia on a synthetic agar. Sclerotia form in tubes and plates in about ten days and occur rather characteristically in concentric rings.

UNIVERSITY OF KENTUCKY
LEXINGTON, KENTUCKY

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THE CONDUCTION OF POTASSIUM CYANIDE IN PLANTS

JOHN A. ELLIOTT

WITH TWO FIGURES IN THE TEXT

During 1914 and 1915, several papers appeared on the effect on trees of treatment with potassium cyanide for the control of sap-sucking and wood-boring insects. At that time the writer conducted some experiments to determine the distribution of and injury due to potassium cyanide (KCN) when introduced into plants. The results of this work were again called to mind by the paper of Rankin.[1] It is thought that the results of the writer are of interest in comparison with those of Dr. Rankin.

Sanford[2] reported that he had, in February, 1914, bored a hole in the bole of a peach tree infested with the cottony-cushion-scale, and by filling the hole with potassium cyanide had completely eradicated the scale. Later[3] he cut down the tree and found that it had been uninjured by the treatment. He was supported by Shattuck[4] who reported that he had many times used potassium cyanide very successfully for killing wood-borers in elm and black locust trees, and without injury to the trees.

On the other hand, Surface[5] reported serious injury done to orchards throughout Pennsylvania by "tree doctors" who were using potassium cyanide, ferrous sulfate, potassium chloride and perhaps other salts for "curing" fruit trees of insect troubles and other diseases. He reported dark staining of the cambium of the trees treated, especially upward from the hole made for inserting the above-mentioned salts. Many trees had been killed while others were less injured. In experiments reported more recently, Moore and Ruggles[6] and Flint[7] obtained results supporting

[1] Rankin, W. H. The penetration of foreign substances introduced into trees. Phytopath. **7**: 5–13. 1917.

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Surface insofar as injury to the trees is concerned. The appearance of the injury reported is also in accord with that found by the writer.

While it is not within the scope of this paper to discuss the nature or cause of the injury, it may be stated here that the injury as found is in accord with what might be expected from the conclusions reached by Loew,[8] who did fundamental work on the effect of potassium cyanide and di-cyanogen on plants and low animals, finding that di-cyanogen is actively toxic to both active and dormant protoplasm while potassium cyanide, in dilute solutions, is toxic only to active protoplasm.

Woods[9] and Townsend[10] experimented with the effect of hydrocyanic acid gas on plants. Townsend reported the effects of the gas on germination of seeds treated under different moisture conditions. Woods found that some plants were more resistant to the gas than others and that the injury to growing woody plants was localized mostly in the young xylem cells next to the cambium.

The results of experiments by the writer disagreed in important points from those given by some of the experimenters cited above. The experiments were planned with the following points in view: (1) The path of conduction of potassium cyanide through the plant. (2) The extent of injury, local and general.

THE PATH OF CONDUCTION OF POTASSIUM CYANIDE IN PLANTS

For the purpose of ascertaining the path of conduction of potassium cyanide in plants, both herbaceous and woody plants were used, the cyanide employed being either in crystal form or in solution.

Tests for potassium cyanide in the tissues of the plants were made with the ferric chloride-ferrous sulphate solution as given by Molisch in Microchemie der Pflanzen. The presence of the cyanide is shown by the formation in the tissues containing it, of Prussian blue. The iron solutions often failed altogether to give a reaction for potassium cyanide, in the tissues of plants, forty-eight hours after the plants had been treated, except at the place of treatment. No positive tests could be made after three or four days. When using herbaceous plants the best tests were secured within fifteen minutes to twenty-four hours after treating; with tree branches the best tests could be made within from one to twenty-four hours.

[8] Loew and Tuskamoto. On the poisonous action of di-cyanogen. College of Agriculture, Tokio, Bulletin **2**: 34-41. 1895.

[9] Woods, A. F. The variable effect of hydrocyanic acid gas on plants and animals. Am. Nat. **32**: 104. 1899.

[10] Townsend, C. O. The effect of hydrocyanic acid gas on the germination of seeds. Bot. Gaz. **31**: 241-264. 1901.

In treating tree branches the crystal potassium cyanide was weighed and inserted into the desired ring of growth by means of glass tubes. A hole just large enough to admit the tube was bored into the branch to the desired depth; then the potassium cyanide, which had been plugged into the glass tube with cork, was pushed out of the tube into the hole by means of a plunger and kept in the desired place by means of the cork which had held it in the tube, and by wax over the cork. In this way the potassium cyanide could be put into the portion of the wood desired and be kept from coming into contact with any other part. Other branches were treated by raising the bark and putting 5 grams of potassium cyanide under it without cutting the bark at the upper end of the raised portion. The wound was then covered with wax.

Contrary to the observations of some of the writers cited above, it was found in testing for cyanide in the tissues by means of the iron solutions, that in all cases the blue staining was in the walls of the vessels and to a slight extent in the cells surrounding the vessels, as would be expected from the solubility of potassium cyanide and our knowledge of the path of ascent of sap. Under conditions affecting the rate of transpiration, there was greatest diffusion into the cells surrounding the vessels when transpiration was slowest. When transpiration was rapid the potassium cyanide was more quickly exhausted and tests for its presence resulted negatively. When the potassium cyanide was put under the bark of a branch without injury to the wood, the subsequent test for cyanide showed that the path of conduction was limited to the vessels in the new growth next to the cambium. Diffusion from the vessels into the surrounding cells was slight. Sections from a shoot of the current year's growth in line of the ascending potassium cyanide gave a reaction for cyanide in the vessels and often in the medullary rays and parenchyma of the bark.

INJURY DUE TO POTASSIUM CYANIDE

The primary injury to plants due to the treatment exactly corresponded to the distribution of the potassium cyanide. The injury, both to woody and herbaceous plants, was ascertained by the same means as has just been described for locating the path of conduction. The plants treated were sunflowers (*Helianthus annuus*), giant rag-weeds (*Ambrosia trifida*) young box elders (*Acer negundo*) in their second year's growth, two potted rose plants (*Rosa* sp.), two large apple trees (*Pyrus malus*), and a willow tree (*Salix alba*).

In the smaller plants the injury due to the potassium cyanide was very marked. On a hot, dry day the wilting of the leaves of both the herbaceous plants and the small woody plants was quite noticeable within ten

minutes and the fumes due to the potassium cyanide were readily detected coming from the leaves. On the sunflower plants dark streaks were soon apparent ascending the stems from the place of inserting the potassium cyanide. Darkened areas also occurred along the veins of the leaves reached by the affected bundles, the area of injury spreading from the veins bearing the cyanide to the mesophyll adjacent to them. Of the three veins entering the leaf of a sunflower or a rag-weed, any one, any

FIG. 1. LEAF OF AMBROSIA TRIFIDA INJURED BY POTASSIUM CYANIDE

two, or all three might bear potassium cyanide, this depending on the bundles in contact with the potassium cyanide in the stem. Usually more than half of the leaves above the place where the potassium cyanide was inserted were not affected and at times only a first leaf directly above the wound was injured. In twenty-four hours or less the whole stem of the herbaceous plant above the wound, or a large part of it, darkened and collapsed. The darkening also extended a short distance downward on

the wounded side of the stem. The leaves or parts of leaves reached by the veins carrying the potassium cyanide soon dried out (fig. 1), while the leaves or parts of leaves not immediately injured remained green and turgid for days or even weeks, although the stem of the plant might be apparently dried out. Plants treated on cool damp days showed little injury for several hours but within twenty-four hours the stems of these plants had usually darkened and collapsed. The injury to the leaves of these plants was less extensive than to those treated on hot dry days.

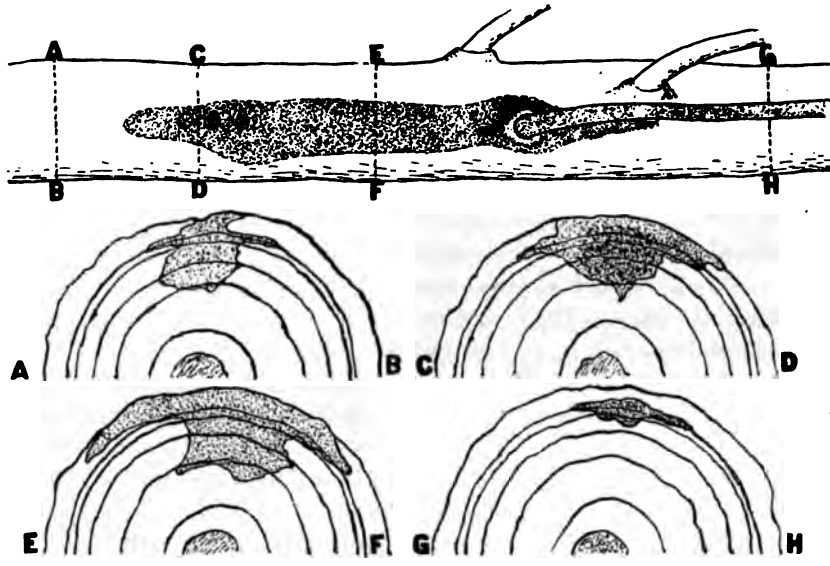

FIG. 2. BRANCH OF APPLE TREE INJURED BY POTASSIUM CYANIDE

The branch was treated with potassium cyanide and the sections made at the places indicated, one week later.

In the smaller woody plants, rose and box elder, the injury was first noticeable in the mesophyll along the veins of the leaves. Within twenty-four hours the bark began to turn yellow and the whole plant gradually dried out above the wound.

Branches of apple or willow trees treated with 5 grams of potassium cyanide under the bark, or treated in the wood of the current year's growth (June), within from twenty-four hours to forty-eight hours showed yellowing of the twigs above the wound on the side of the branch in which the potassium cyanide had been placed (fig. 2). In a week or a little more a narrow strip of bark extending from three to seven feet above the wound to from one to six inches below would darken and collapse. Removing

the bark revealed dead darkened cambium on an area slightly more extensive than showed externally by the sunken bark. When the potassium cyanide was put into the wood of the current year's growth and deeper, more of the wood was involved in the injury, but the injury never extended to the rings of growth which were not in contact with the cyanide. Where the potassium cyanide was successfully plugged out of the current year's growth there was no external appearance of injury to the branches, although the wood cells were killed wherever the cyanide reached them in sufficient concentration. The cambium and other active living tissues were readily killed by the cyanide and such injury necessarily had a serious effect on the plant as a whole, the death of the plant following if a sufficient amount of the cambium were killed. When the dead cambium girdled the plant or branch, all parts above necessarily died.

SUMMARY AND CONCLUSIONS

1. Conduction of potassium cyanide in plants. The potassium cyanide is conducted in the vessels and diffuses from them into the surrounding tissues. The diffusion is greatest when conduction is slowest.

2. Extent of injury. The injury to the plants is local, corresponding to the distribution of the potassium cyanide, except where the local injury has a secondary injurious effect on other parts of the plant. Actively growing tissues are most readily killed. The injury to woody plants is dependent on the amount of cambium reached by the cyanide and the extent to which other parts of the plant are dependent on the injured cambium.

The wide differences in results of treatment of plants with potassium cyanide as have been reported were undoubtedly due to slightly different manners of treatment in the different cases. From Loew's results it is to be expected that less injury would result to trees treated in the dormant state than in active growth. The rapid disappearance of potassium cyanide from the tissues might explain some of the failures to locate its path of conduction.

ARKANSAS EXPERIMENT STATION
FAYETTEVILLE, ARKANSAS

REVIEWS

Plant Materials of Decorative Gardening. The Woody Plants. By William Trelease, Professor of Botany in the University of Illinois. Published by the author, Urbana, Illinois, 1917. $1.00.

This pocket manual will be of great value to collectors of pathological material, the more so since it includes the cultivated and introduced species commonly met with.

W. A. ORTON

PHYTOPATHOLOGICAL NOTES

The perennation of Cronartium ribicola Fisch. on currant. During the summer of 1915 a block of black currants (*Ribes nigrum*) in a nursery were severely affected with *Cronartium ribicola* Fisch. and considerable defoliation occurred. In 1916 the rust appeared again and the same bushes were completely defoliated in August. The bushes were condemned as unsalable stock in the autumn of 1916. The month of July and the first half of August were without rain, but fall rains began on August 17. Late growth occurred on such nursery stock as plums, peaches, etc., but the currants were not seen after defoliation in August. Two hundred of the bushes were dug in October and placed in a nursery storage cellar, where they remained throughout the winter under the usual storage conditions for nursery stock. In the spring of 1917 the bushes were shipped to Ithaca, New York, and planted in an open field. (The nearest known infection of *Cronartium ribicola* is about forty miles from Ithaca.) All of the bushes grew and the new foliage was frequently examined throughout the summer for lesions of the rust fungus, but no signs of the disease had appeared when the bushes were examined for the last time, October 9, 1917.

Some question has been raised concerning the results of experiments reported by F. C. Stewart and W. H. Rankin,[1] who obtained rusted currant bushes in the autumn of 1912 and planted them in the greenhouse. During the winter the bushes developed new foliage, but no infections of *Cronartium ribicola* appeared. It has been suggested that possibly the artificial conditions which obtained in the greenhouse experiments of

[1] Stewart, F. C., and Rankin, W. H. Does Cronartium ribicola over-winter on the currant? New York (Geneva) Agr. Exp. Sta. Bul. **374**: 41–53. 1914.

Stewart and Rankin were not conducive to the germination of the rust spores or to the development of the fungus in the host tissue. On the other hand, the above-mentioned two hundred black currants planted by the writer in 1917 were subjected to the usual nursery methods, the currants being dug in the autumn and placed in storage until spring. If the fungus hibernates in infected bushes or on old diseased leaves which may cling to the bushes under these conditions, at least a certain portion of the two hundred plants should have shown the disease after the new foliage developed. Weather conditions were exceptionally favorable for the fungus throughout the summer.

V. B. STEWART

The occurrence of Colletotrichum cereale, Dothichiza populea, and Leptosphæria napi in Canada. The wheat anthracnose described by Selby and Manns, caused by *Colletotrichum cereale* Manns, has been found in Charlottetown, Prince Edward Island, where it apparently is similarly injurious and otherwise identical in character and nature.

Dothichiza populea Sacc. & Briard has caused the death of and injury to several Lombardy poplars at St. Andrews, N. B. These two diseases have so far not been recorded from the Dominion of Canada.

A third disease is of interest at this time where the raising of diverse seed supplies is considered very important. The fungus is *Leptosphæria napi* (Fuckel.) Sacc., which often carries great destruction to turnip and rape seed cultures in Germany. The disease was observed on seed pods of turnips at Charlottetown, P. E. I., and appears to be similarly destructive in Canada to the production of turnip seed as in Europe. It should not prove difficult to prevent serious injury to a crop by spraying in time and at regular intervals.

H. T. GÄSSOW

Cronartium cerebrum on Pinus resinosa. It is common observation that the Norway pine (*Pinus resinosa*) is very free from the attack of forest tree rusts. This is all the more interesting in view of the fact that most all the hard or yellow pines are either known hosts or possible hosts for a number of rusts. The literature to date records but three species of rusts on *Pinus resinosa*, two foliicolous and one caulicolous forms.[1]

On June 8, 1912, in a part of the Minnesota National Forest near Cass Lake, a young Norway pine was found bearing four galls which on subse-

[1] Pierce, R. G. Pinus resinosa, A new host for Peridermium acicolum. Phytopath. 6: 302. 1916.
Spaulding, P. Notes on Cronartium comptoniæ. III. Phytopath. 7: 49. 1917.
Spaulding, P. Needle Rust of Pinus resinosa. Phytopath. 7: 225. 1917.

quent examination proved to be caused by *Cronartium cerebrum* (Pk.) D. & L. The infection was recent, the galls being borne near the ends of four different branches. The galls were small and had greatly retarded the growth of the branches. The tree stood in a dense stand of jack pine (*Pinus banksiana*) which was heavily infected with the same rust. A diligent search at the time resulted in finding no other infection on *P. resinosa* in the same region. During subsequent years the search for this fungus on *Pinus resinosa* in various parts of the Lake States has been without result. In this connection it is interesting to quote from a letter of July 14, 1917, from H. C. Hilton, Supervisor of the Michigan National Forest, as follows: "I have never found a gall Peridermium on Norway pine although it is found associated with infected jack pine all over the lower peninsula." This indicates, so far as field observations go at present, that the Norway pine is a fairly safe tree to plant in all regions where it may find suitable growing conditions.

JAMES R. WEIR AND ERNEST E. HUBERT

A thumb clip for use with magnifiers. Necessity in most cases—convenience in others—is the mother of invention. The illustrations present this useful accessory in a very concise manner. The idea suggested itself

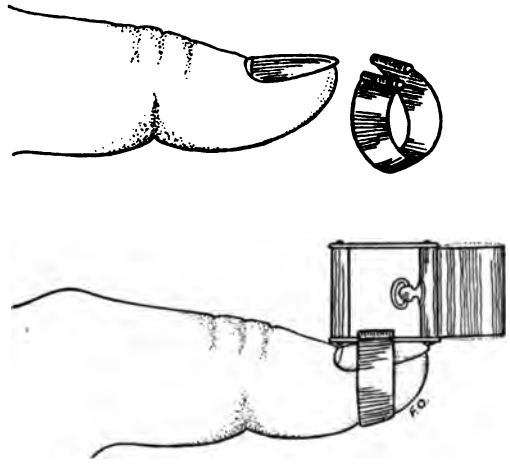

FIG. 1. A THUMB CLIP FOR USE WITH MAGNIFIERS

to me some time ago when engaged in removing individual fruiting bodies of fungi causing leaf spots. The lens was at first fixed in position by means of a rubber band, which had the disadvantage of slipping off just at the critical moment. Later on a small, circular metal strip was cut

and bent to fit the thumb and securely clip the lens—an ordinary 20 diameter Coddington. Other similar makes have been tried with equal success. The clip requires a certain amount of spring to it, which has been secured by the turned up brim of the clip.

The advantages are obvious. The right hand is free to operate scalpel, forceps, or needle, the fingers of the left hand to hold the object, and the thumb carries the lens which is very easily held at the right focal distance.

FIG. 2. THUMB CLIP IN USE

Even a very minute object may thus be removed with ease and certainty. In use nearly every day it revealed additional advantages. No doubt entomologists as well as plant pathologists will find this little accessory as useful as has its "inventor."

H. T. GÜSSOW

Tylenchus tritici on wheat in Virginia. Specimens of the disease were received from a correspondent at Dovesville, Rockingham County, Virginia, on August 22, 1917. An examination of the shriveled, blackened grains (fig. 1) found in the discolored heads disclosed the presence of the larvae of a nematode in great abundance. The correspondent stated that he had sown the same seed wheat for about ten years. At first only an occasional head was affected, but within the last six years or so the in-

fection has become so general that he has searched for a remedy. Thinking it a form of smut, he tried both the formaldehyde and hot water treatments, but with no noticeable measure of success. He estimates the loss this year at about 25 per cent on ten acres. Johnson[1] reported this

FIG. 1. GRAINS OF WHEAT PARASITIZED BY TYLENCHUS TRITICI AND NORMAL GRAINS

disease on wheat from California, Georgia, West Virginia, and New York in 1909. Byars[2] has recently called attention to its presence in wheat received from China, but states that he knows of no record of it from the United States before or since Johnson's records.

F. D. FROMME

Field conference of cereal pathologists. The Third Annual Field Conference[3] of Cereal Pathologists of the American Phytopathological Society was held at Madison, Wisconsin, on July 9, 10, and 11. About forty were in attendance at the various meetings.

The three days were occupied in field trips into the grain growing sections about Madison and in round-table discussions on the various phases of cereal pathology. Among the subjects discussed were:

1. *Grass rusts and their rôle in cereal conservation*, led by Dr. J. C. Arthur and Dr. E. C. Stakman.

2. *The relation of the barberry to rust epidemics*, by Dr. E. C. Stakman and Mr. Frank Piemeizel.

3. *State and Federal legislation against the barberry*, by Prof. H. L. Bolley.

4. *State and Federal cooperation in fighting cereal diseases during our food emergency*, by Dr. H. B. Humphrey and Dr. S. G. Kern.

5. *Recent investigations on yellow stripe rust*, by Dr. Charles W. Hungerford.

[1] Science, n. s. **30**: 576. 1909.

[2] Phytopathology **7**: 56. 1917.

[3] A more complete account of this meeting has been published in Science, n. s. **46**: 316–318. 1917.

At the various meetings resolutions were passed, as follows:

I. To the Honorable The Secretary of Agriculture.

We, the plant pathologists representing the chief grain-growing states in conference assembled, in recognition of the following facts:

1. The national and international need of the maximum production of all food grains for the immediate future,

2. The preventable losses resulting from smuts and other seed-borne diseases

3. Practical and simple methods of seed treatment known to prevent such losses.

4. The Office of Cereal Investigations has already instigated a movement looking to the more universal treatment of seed for the prevention of these losses,

Resolve: (1) That it is our conviction that this work should be pushed with all possible diligence; (2) That we as representatives of these grain-growing states pledge to this work our hearty cooperation and support.

II. In view of the vital importance of the wheat crop and as a national emergency measure likely to prove an effective aid in increasing and insuring a better wheat crop in 1918, *be it resolved:* That we, the cereal pathologists of the American Phytopathological Society, in summer session assembled at Madison, Wisconsin, respectfully ask the President of the United States to appoint a commission to consider the relation of the barberry to outbreaks of black stem rust of wheat, barley, other cereals, and grasses, with a view of deciding upon the desirability of eradication of all cereal rust-bearing strains of the barberry in the United States in order that this source of rust epidemic may be removed.

Be it further resolved that the Secretary be instructed to send a copy of this resolution to the President of the United States.

III. *Moved and carried:* That the chairman of this body appoint a committee to take up with federal authorities the matter of securing some definite action to insure an adequate supply of fungicides and insecticides, particularly those containing copper, for the protection of important crops against the destruction of fungous diseases and insect pests and to insure a reasonable price for the same such as shall not be prohibitory to their use by the farmers and fruit growers of the United States.

Personals. Dr. R. A. Jehle, who was formerly in the service of the Florida Plant Board, and Dr. Geo. K. K. Link, of the University of Nebraska, have accepted appointments as pathologists in the Bureau of Plant Industry, in connection with extension work on the control of truck crop diseases.

Mr. Wm. N. Ankeney, formerly an assistant in the Department of Botany of Ohio University, who has been employed during the past summer as field assistant in cucumber disease investigations, Bureau of Plant Industry, will continue this work under a recent permanent appointment as scientific assistant.

Mr. J. C. Walker of the Department of Plant Pathology, University of Wisconsin has been appointed scientific assistant in the Bureau of Plant Industry, to take up work on the diseases of onions and other truck crops.

Mr. William W. Diehl, recently instructor in botany at Clemson College, South Carolina, and formerly a graduate student in botany at Iowa

State College, has been appointed scientific assistant in the Office of Pathological Collections and Taxonomy of Fungi, Bureau of Plant Industry.

Mr. Max W. Gardner of the University of Wisconsin, who has been employed during the past three summers as field assistant in cucumber disease investigations, Bureau of Plant Industry, has accepted a position as instructor in plant pathology in the University of Michigan. Mr. I. C. Hoffman of Purdue University, who has been engaged in the same line of work, has become assistant in the Department of Horticulture of the Colorado Experiment Station.

Mr. D. C. Neal of the Alabama branch Experiment Station at Loxley has accepted an appointment in the Bureau of Plant Industry to continue his investigations of citrus canker.

Mr. Herbert F. Bergman, formerly assistant professor of botany in the University of Minnesota, has been appointed scientific assistant in the Office of Fruit Disease Investigations, Bureau of Plant Industry.

LITERATURE ON PLANT DISEASES[1]

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

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All authors are urged to coöperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles published outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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and work together for the best interest of the fruit grower

The following quotation from a letter just received is typical of the attitude of the U. S. Dept. of Agriculture and many of our Experiment Stations for the past ten years:—

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