INVESTIGATIONS ON THE BLACK ROOT OF STRAWBERRIES

Thesis for the Degree of M. S.

Forrest C. Strong

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Strawberries – Diseases & Pests

Tilletia Blackii root of Strawberries

Botany

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INTRODUCTION

The strawberry industry in Michigan is of considerable importance. In 1924 the U. S. Department of Agriculture reported 5,500 acres devoted to the culture of this fruit. The same year Michigan shipped 554 carloads of berries, ranking tenth in the commercial production of strawberries in the United States. There are four phases of the industry: 1st, the commercial production of berries for the nearby large city markets; 2nd, the growing of berries for the canneries, in which Michigan ranks first in the United States; 3rd, the commercial production of strawberry plants by the nurseries; and 4th, the growing of the berries in the small plantation for the home. The largest acreage set out for commercial production of the fruit and plants is located in southwestern Michigan in Berrien and St. Joseph counties, while other smaller centers are located in Wayne, Monroe, Montcalm, Kent, Van Buren, Allegan, Grand Traverse, and other counties. Local plantations are to be found through the entire state.

The varieties most commonly grown are the Senator Dunlap, Brandywine, Gibson or Pokomoke, and Premier, although others may be more popular in vicinities. Also several everbearing varieties are grown, the Progressive probably being most used.

THE STRAWBERRY DISEASE SITUATION

Among the cultivated crops the strawberry has long been considered one in which the disease hazard for the grower is small. Mildew,

leafspot, and a few fruit rots have at one time and another attracted the attention of growers. For the most part these diseases are of minor importance, and no control measures are practiced.

There is, however, a situation in strawberry culture which is a subject of concern to the growers. This is the failure of plants newly set out to become established, the dying of plants, often those the first year, which in severe cases may involve a considerable area in a plantation. Such dead areas may be so numerous as to render the plantation unprofitable after two or three years.

The following case which came under the writer's observation is typical of the situation commonly found in strawberry fields in the state. In the spring of 1925 a new plantation of 20 acres was set at Edmore in Montcalm County. The first stand was so poor that a second and in some places a third setting was necessary to produce a full rowed stand. Figure 1 illustrates the condition of the field in the spring of 1926, and at this time many plants were dead or diseased. The manager of the plantation stated that the securing of a good stand was becoming increasingly difficult. A small plantation near Bridgeman gave a similar history. This field, although set under fairly normal conditions and in the customary manner, gave a very unsatisfactory stand. Figure 2 shows a typical area in this plantation.

It has long been known that strawberry plantations can rarely be kept at high productivity for more than two years. The standard practice in strawberry growing is to plant in the spring or late summer. The first crop is harvested the following spring. Runners from the

mother plants are there allowed to strike between the original rows, and these original rows are then plowed up. The third year it is commonly found that the stand is so poor and choked with grass that the plantation is on the down grade in production. The problem presented in the running out of the patches is not one of soil fertility, for the strawberry seemingly does well on soils low in fertility, and many plantations on rich soil show poorer stands than those in light soil.

It is suggested that along with drought injury and winter killing, the disease which is the subject of this article is an important factor in the difficulties which have been met with in securing and maintaining a stand of strawberries. As the discussion proceeds, it will be seen that we are concerned with a root disease which by its weakening effect on plants, renders them low in vitality and subject to injury by various factors such as drought and frost, and the conditions to be described will be seen to play an important role in the deterioration of strawberry plantations in this state.

THE BLACK ROOT DISEASE

The aspect of a strawberry plant affected with black root is typical. The diseased plant shows poor vitality, is characteristically stunted, and has tendency to dry up at the onset of dry weather conditions. The plant shown in Figure 3 died two days after the photograph was made. Such a plant on being dug up is found to have a root

system in which the roots are all black, corky in texture, and apparently dead. If a diseased plant as yet not in the wilting stage is examined, there will be seen young white lateral roots pushing out from roots with blackened cortex tissue (Figure 4). Upon examination of such a root the diseased cortex will be found to peel off readily leaving the vascular cylinder, from which the secondary roots arose, still more or less white and apparently functioning. It should be borne in mind that roots dying from age present much the same general appearance as that just described. The examination of a younger plant which is affected with black root yields a more clean cut picture of the black root disease. Even these younger plants show characteristic evidences of the disease by the production of smaller leaves and fewer main roots, together with the reduction in number of lateral roots. Young plants affected with black root lack vigor as is manifested by their slow growth and reduced runner production. Some of the main roots of such plants will be rotted their entire length and brown to black in color. From few to many lesions are to be found on the main roots, and many or all of the lateral or secondary roots will either be rotted off or show distinct rotted areas (Figure 5). The lesions on the main roots vary in size from .5 to 5 cm. in length and usually encircle the root. At first they are reddish brown and later become black. These lesions when in the early stage exhibit a brown water-soaked appearance but very soon become shriveled and shrunken. Very often the rotting involves all of the root tissue, affecting the stele or vascular cylinder as well as the cortical tissue (See Figures 6 and 7). Such roots

break at the rotted portion. The lesions on the laterals resemble in minature those on the longer roots. It is probable that the attack upon the lateral roots is very important in producing the general effects of black root, since the root hairs through which water and plant food materials enter the plant largely occur on these portions of the root system. The loss of these lateral roots then reduces the efficiency of the root system. This lack of a properly functioning root system is seen in plants affected with black root or root rot during dry periods of summer and especially during the picking season if it is at all dry. On a hot day the leaves will wilt down and then recover during the night. If the dry weather continues, the plants will die with the berries hanging green and ripe on the stems, while the leaves may become purplish or bronzed and the petioles turn red.

PREVIOUS WORK

The writers on strawberry culture have for a long time referred to black root as a diseased condition whose cause was unknown. There is little to be gained by referring to these scattered notes, since none of the older writers made more than casual comment on the severity and probable cause.

What may be a typical case of black root was described by Clinton (3) under the heading "Leaf Scorch", but he stated that no fungus appeared to be affecting the roots. This condition may have been due to black root. Fletcher (7) in 1917 describes "root rot or black root" of strawberry and states that the disease was prevalent in

New York, Michigan, and Massachusetts in the years 1902--1908. "When the berries are about half grown the plants wilt and turn yellow; the roots are decayed. Most of this trouble is due to winter injury, but a bacterial disease is associated with it in some cases. Poor culture, lack of fertility, the crowding of plants in the row, insufficient mulching, and wet land are favorable for this trouble." Heald (8) in 1920 mentioned the "dying out" of strawberry beds in western Washington, and ascribed the trouble to Rhizoctonia. The next year Smith and Horne (11) in California described a rot of strawberries in which the cortex of the root decays. No parasite was found and the rot was believed to be due to water logging of the soil or sudden drying out of soil moisture. The summer of 1923 the disease was reported from Mississippi by Neal (9) who wrote "A root rot of strawberry has been discovered in many parts of the state the present season. The disease causes a distinct wilting of the plants, accompanied by pronounced discoloration of the roots and crown tissue. A species of Fusarium has been isolated from the affected crown, but at present it is not known if this fungus is parasitic or responsible for the death of the plants. This trouble is serious and is causing many growers of strawberries considerable alarm."

Investigations on black root date from this time and are not very extensive. In 1923 Berkeley and Jackson (1) reported the isolation of various soil bacteria which they thought to be the causal organisms. Later, in February 1924, Berkeley (2) further reported that black root was present again the second season and did much damage.

The soil bacteria isolated previously failed to cause infection when tested by inoculation into plants. A species of Fusarium was suspected and mycelium was found in sections of diseased roots. In 1924 Sherbakoff (10) reported black root as a disease of wide occurrence in the southern states and also further north. He stated that it is of considerable economic importance in certain sections. He isolated a fungus from the diseased roots but was unable to identify it because it remained sterile in culture. Coons (4) also in 1924 described black root of strawberries in Michigan and stated that the conditions of infection indicate Rhizoctonia as the causal organism. In 1926 Darrow (5) summed up the situation as follows: "There are severe troubles known as black root which are caused by fungi or bacteria. No thorough studies of these troubles have ever been made, but their effect is to kill the roots and so shut off the intake of water and dissolved minerals." Again--"Little is known of the cause or control of crown or root diseases of strawberries. They are often very serious and may cause serious injury which is not recognized as due to disease. They may even be the most important of all strawberry diseases." Late in 1926 Wardlaw (12) reported on the Lanarkshire disease of strawberries in Scotland. His description is identical with the black root disease in this country. He isolated a Pythium which upon inoculation produced typical symptoms of disease.

OCCURRENCE OF THE DISEASE

The black root of strawberries is widely distributed not only in the United States, but abroad. What appears to be a similar dis-

ease has been reported from Scotland and Africa.* In the United States and Canada, as already shown in the preceding section, the disease is known from Mississippi, Tennessee, Alabama, New York,** Ontario, California, and Washington. On the trip made by the 5th Annual Meeting of the American Phytopathological Society in 1923 through western New York state and Ontario, Canada, the disease was observed at various places.

Collections in Michigan have shown the disease to be widely distributed, specimens having been obtained from Berrien, Ingham, Wayne, Monroe, St. Joseph, Allegan, Montcalm, and Van Buren counties. It is evident that this disease is extremely wide spread and coincident with the culture of strawberries. Since strawberry plantations are established with nursery plants and new varieties are constantly being introduced by nurseries, one might suspect that we are dealing with a disease of strawberries distributed perhaps on nursery stock and thus becoming introduced and established in the fields.

Opposing this view is the fact that wild plants have been found either killed out entirely or showing marked evidences of the disease by the presence of lesions and rotted roots. These wild plants have been found in many places in the state, and many cases have been observed in locations where strawberries have never been grown as a crop. It will be remembered here that wild strawberry patches arise from seedling plants, the seeds having been carried by

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* Verbal communication to Dr. G. H. Coons by Dr. E. J. Butler Imp. Bur. of Mycol.

** Verbal communication to Dr. G. H. Coons by Dr. H. E. Thomas of Cornell Univ.

birds. This situation together with the wide spread distribution of the disease strongly indicates that the disease occurs more or less irrespective of soil types, and the cause is to be sought among the naturally occurring soil organisms.

THE NATURE OF BLACK ROOT

As has been said, examinations of the roots of plants affected with black root showed rotted roots and various sized browned and blackened areas, as illustrated in Figures 6 and 7. These lesions indicate a parasitic organism as the cause.

The black root of strawberry had every earmark of a parasitic disease. It has been pointed out that the situation was confused by drought and winter injury. In order to determine whether we were concerned with a disease caused by some parasitic organisms and not by some soil or climatic factors, a series of preliminary experiments were performed.

Dead and dying plants typical of the black root conditions were dug up and their root systems chopped into small pieces. This mass of debris was placed in pots so that strawberry plants could be grown with new roots passing through this material. A series of 19 pots were filled two-thirds full of clean sand, a one inch layer of debris added, and the pot then filled with sand. As a control in this experiment, pots of clean sand with no black root inoculum were employed. Also pots containing a layer of debris which had been sterilized by treating at 15 lbs. pressure for 30 minutes in an auto-clave were used as additional controls. Young runner plants with root tips

TABLE I.

EFFECT OF BLACK ROOT MATERIAL ON YOUNG ROOTS OF STRAWBERRY:

Runners rooted (a) in clean sand plus black root material, (b) in clean sand plus autoclaved black root material, (c) in clean sand.

Inoculated				Control			
(a) Clean sand plus black root material				(b) Clean sand plus autoclaved black root material		(c) Clean sand	
Plant No.	No. of lesions	Plant No.	No. of lesions	Plant No.	No. of lesions	Plant No.	No. of lesions
1 a	2	17 a	5	1 b	0	1 c	0
2	3	18	4	2	0	2	0
3	6	19	6	3	0	3	0
4	2	20	2	4	0	4	0
5	0	21	3	5	0	5	0
6	4	22	3	6	0	6	0
7	2	23	3	7	0	7	0
8	6	24	1	8	* ?		
9	0	25	4	9	0		
10	2	26	6	10	0		
11	2	27	3	11	0		
12	2	28	1	12	0		
13	2	29	4	13	**3		
14	7	30	4				
15	4	31	9				
16	0	32	2				

* Plant 8 (b) had one suspicious brown stripe
** Plant 13 (b) had 3 typical lesions.

just showing were set into these pots. Frequent waterings were made to keep the sand and debris inoculum moist. Examinations and readings of the condition of the roots were made in about 14 days. The result of the test as shown in Table I was conclusive. In this experiment out of 32 inoculated plants typical black root appeared on 29 while in the sand controls, 7 plants remained healthy, as evidenced by the white roots. The 14 plants inoculated with sterilized debris showed 11 plants healthy, 3 plants were noted as suspicious since they had roots with slight discoloration which might be confused with true infection.* The great number of lesions appearing on the roots of inoculated plants as compared with the clean plants in the sand controls as well as the plants inoculated with sterilized debris indicates quite clearly that the disease is infectious and transmissable from one plant to another and parasitic in nature rather than attributable to some soil factor or to winter injury. Examination with the microscope of sections made from diseased roots gave abundant evidence of septate hyphae in the root tissues.

ISOLATION OF THE CAUSAL ORGANISMS

Many plantings were made of bits of blackened roots in petri dishes containing nutrient medium. Material for use in making such plantings was procured partly from strawberry plants sent in to the Botanical section of the Experiment Station for disease diagnosis, and partly from plants collected by the writer and others of the Botanical

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* A plausible explanation of this might be that it is natural infection due to presence in a diseased field and in an open pot.

Staff in various parts of the state where strawberries are grown extensively.

Prune agar was commonly used as the medium for isolation purposes since its slightly acid reaction discourages bacterial growth. Potato dextrose agar, cornmeal agar, and strawberry root agar were also used. The technique of making these plantings was to wash the roots carefully to remove the dirt. The roots were dried of excess moisture on sterile paper toweling, split lengthwise, the splitting being merely initiated by sterile needles or forceps. Plantings were made from the interior tissues now exposed and which had not been touched by anything, in order that no contamination might be introduced. Washing the roots with disinfectants was unsatisfactory since no growth resulted from plantings made from materials so prepared.

The range of material employed and the number of plantings made are believed by the writer to be extensive enough to afford a fair sampling of black root material as it occurs in Michigan and to indicate the organisms chiefly involved. The isolations from diseased tissue were extended over a period of nearly three years and involved the making of more than 2,300 isolations from diseased plants collected in various parts of the state. The fungi appearing most often in these plantings were: a species of Coniothyrium, a species of Gloeosporium, and a species of Fusarium (brown). The first two appeared with great frequency, the Fusarium appearing more often in earlier isolations than in the later ones. Other organisms appearing occasionally were:

Acrostalagmus sp., ared Fusarium, Pythium sp., as well as various species of Mucor and of Penicillium.

TESTS OF SUSPECTED ORGANISMS FOR PATHOGENICITY

Following the isolation of Coniothyrium sp., Gloeosporium sp., and the brown Fusarium in plantings from diseased tissue, these organisms were secured in pure cultures and tested by repeated transfers. Several series of inoculation experiments were set up, using them as inocula to test their pathogenicity.

TESTS WITH FUSARIUM (BROWN) SP.

Inoculation of Strawberry Runner Plants Grown on Agar

The first inoculation experiments were made in the greenhouse. Runner plants were set in 500 and 1000 cc. Evlenmeyer flasks which contained a solution of nutrient medium solidified with agar. This medium covered the bottoms to the depth of about 2.5 cm. Following a 10 minute treatment in a 2--4% chlorozene solution to give surface disinfection and a rinsing with sterile water, each runner plant was introduced into a flask so that the node where the root tips were showing just touched the surface of the medium. Roots 3 to 4 inches long developed in about two weeks. These roots were inoculated by introducing bits of mycelial mats into the agar close to the roots. The runners remained attached to the mother plants throughout the entire period of the experiment.

In three series of inoculation experiments using the brown Fusarium, infection amounting to an average of 44% resulted. Plantings were made from bits of roots browned by contact with the inoculum, but recovering of the Fusarium organism was not always successful. Infected areas on the roots inoculated were not very typical of the black root disease. Although a certain pathogenicity was indicated, decision is reserved as to the importance of this organism in the black root disease.

TESTS WITH CONIOTHYRIUM SP.

Inoculation of Strawberry Runner Plants Grown on Nutrient Agar

Coniothyrium sp., which was characterized by the production of black pycnidia, showed up with considerable frequency on the isolations made from diseased plants. This species produced infection on 4 out of 5 plants grown on agar in a preliminary inoculation experiment. The rotting of roots and lesions were typical of Black Root disease as found in the field.

Inoculation of Strawberry Seedlings on Sterile Sand

This same organism was used to inoculate strawberry seedlings* grown in deep culture dishes on sterile sand. In this experiment, out of 24 seedlings inoculated, 21 were infected. See Table II. In the controls consisting of 42 plants, 2 were dead. These 2 plants showed

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* Akenes of strawberries ripened the previous summer were disinfected and sowed on sterile sand in deep culture dishes 6"--8" in diameter. Due to the fact that the strawberry akene has a hard coat, it is possible to treat it in concentrated sulphuric acid for periods of 8--10 minutes without injury to the young embryo. De Zeeuw (6) has shown that this is the best way to sterilize such seeds as are not injured by concentrated sulphuric acid. The percentage of germination is also increased. Success in the use of this method lies of course

no fungi externally or internally. This is thought to be the natural death of the plant due to lack of nutrition. Some time after the death of the plants infected, pycnidia appeared on the roots, stems, and seed leaves. These pycnidia were submerged in the tissue of the host. Plantings were made of diseased seedlings, and the Coniothyrium was recovered in pure culture.

TESTS WITH GLOEOSPORIUM SP.

Inoculation of Strawberry Seedlings on Sterile Sand

A brown acervulus forming organism identified as Gloeosporium sp. also showed up quite consistently on plate isolations. In preliminary inoculation experiments with this organism on strawberry seedlings grown in deep culture dishes on sterile sand, 20 infected plants resulted from 21 inoculated. The same controls were used as for the inoculation with Coniothyrium sp. in the preceding section.

Infection takes place in shorter time than in the field due probably to the ideal growth conditions for the fungi. The results of the several series of seedling inoculations are arranged in summarized form in Table II.

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in the rapid addition of sterile distilled water after the removal of the sulphuric acid. If addition of water is slow or the quantity of water is not sufficient, heating will ensue. By using sterile pipettes and growing in sterile sand cultures, the writer has been successful in growing sterile seedling plants for as long as six weeks without the entrance of any contamination. The seeds germinate and start growth at once. They will grow to a height of about 2 to 3 cm. in from 8 to 15 days. When about this height and before they have to rely much on their own food manufacturing powers, they were inoculated.

TABLE II

SUMMARY OF SEEDLING INOCULATIONS.

Inoculated with Gloeosporium sp.			Inoculated with Coniothyrium sp.			Control		
No. of Plants	Time	Plants Infected	No. of Plants	Time	Plants Infected	No.of Plants	Time	Plants Infected
			12	13 days	12	46	13 days	0
21	11 days	20	24	13 days	21	42	11 days	2 *
195	21 days	184	383	21 days	363	111	21 days	16 *

* **These plants showed no fungi externally or internally. This is thought to be the natural death of less vigorous plants due to lack of nutrition.**

FURTHER EVIDENCES OF PATHOGENICITY

of

Coniothyrium sp. and Gloeosporium sp. for Strawberry Roots.

The next series of inoculations were undertaken in the field where runner plants were set in pots filled with clean sand, (Figures 8 and 9) the purpose being to secure the natural conditions of plant growth and yet avoid as much as possible the contamination from the soil in which the mother plants were growing. In this way it was sought to demonstrate for the two organisms shown to be capable of producing black root under laboratory and greenhouse conditions, the production

of disease comparable to the field form of black root. The runner plants remained attached to the mother plants throughout the entire period of the experiments. The runner plants were selected before any roots had started, so that all root production was made in the clean sand. The inocula consisted of pure culture material of the Coniothyrium sp. and the Gloeosporium sp. grown on various media, prune agar, potato dextrose agar, cornmeal mush, and sterile rotted manure. The chief method of inoculation was to introduce pure culture material of these organisms into the sand in the pots where the runner plant roots were growing.

Early in these series of inoculations, spore suspensions were used. Later mycelial masses were used in small quantities, and it was found that inoculations giving the highest per cent of infection and in the shortest period were those in which large pieces of mycelial mats were laid against the roots.

At intervals of about 2 and 4 weeks the plants were removed from the pots and examined. They were then either carefully reset or were brought to the laboratory, photographed, and reisolations made from the lesions.

The inoculation tests of August 2, 1926, gave the most decisive results of any, for in this series lesions in the control plants were absent. The results of this experiment which followed the general technique just described are given in Table III, and show after a 12 day period in the case of 13 plants inoculated with Gloeosporium sp. 100%

TABLE III

ACTUAL RESULTS OF INOCULATION OF CLEAN RUNNER PLANTS, AUG. 2, 1926.

10-12 day reading

Gloeosporium sp.				Coniothyrium sp.				Controls			
Plant NO.	Lesions on lateral roots	Lesions on main roots	Rotted roots	Plant No.	Lesions on lateral roots	Lesions on main roots	Rotted roots	Plant No.	Lesions on lateral roots	Lesions on main roots	Rotted roots
1	1	3	0	1	13	9	0	1	0	0	0
2	2	6	2	2	12	4	0	2	0	0	0
3	0	1	0	3	4	2	0	3	0	0	0
4	8	5	1	4	8	4	0	4	0	0	0
5	0	0	0	5	4	7	0	5	0	0	0
6	4	3	0	6	5	7	0	6	0	0	0
7	6	2	0	7	12	1	0	7	0	0	0
8	8	5	0	8	13	11	0	8	0	0	0
9	0	1	0	9	3	3	0				
10	13	6	0	10	15	6	0				
11	11	2	0								
12	2	0	0								
13	3	0	0								
Total	94 lesions			Total	143 lesions			Total	0 lesions		

infection either in the form of lesions on main roots or on laterals, or severe effects leading to rotting of the entire root. Similar results were obtained with Coniothyrium sp. except that no roots were completely rotted in the period of this test.

During the course of this investigation, many such tests were made, all of them giving conclusive evidence of the pathogenicity of the two organisms; but in many of these cases, especially with long periods of observation, a small amount of disease developed naturally in the controls. The data of these tests being too extensive to be given in full are summarized in Table IV a, b, and c.

The type of experiment possible under field conditions, if one desires to work with plants as nearly normal as possible, is one in which a medium free from infectious organisms is used for rooting the runners. The possibilities of chance introduction of organisms on the runner plant and from dust is considerable, and proper conclusions from experiments with soil organisms, such as those considered here, must be based upon the relative amounts of infection in inoculated plants and the similarly handled controls.

It will be noted from a study of Table IV that in general the control plants show a small amount of infection which is to be regarded as casual infection which practically cannot be avoided in field work of this nature. Comparison of this infection in the control plants with the far greater infection found in the inoculated plants as shown in columns 7 and 8 of Table IV a and IV b the writer believes to be conclusive evidence of the pathogenic nature of the two organisms.

TABLE IV a

SUMMARY OF INOCULATIONS OF CLEAN RUNNER PLANTS WITH GLOEOSPORIUM SP. SUMMER 1926

Date of Inoculation	Total number of plants	Number of infected plants	Total number of lesions	Average lesions per inoculated plant	Average lesions per control plant	Percent infection due to inoculation	Ratio of increase in infection in inoculated plants
July 10th 1st series 27 day reading	40	27	104	2.6	.66	75%	4:1
July 10th 2nd series 10 day reading	14	5	22	1.6	.32	80	5:1
July 10th 2nd series 27dday reading	15*	12	104	6.9	.66	90	10:1
July 21st 13 day reading	54	5	11	.2	.05	75	4:1
July 21st 34 day reading	54	43	350	6.5	5.30	18	1.2:1
July 27th 10 day reading	25	13	72	2.9	.05	98	58:1
August 2nd 10 day reading	13	12	94	6.4	0	100	---
August 2nd 29 day reading	13	13	113	8.7	2.50	71	3.5:1
August 4th 1st series 8 day reading	19	16	117	6.0	0	100	---
August 4th 2nd series 27 day reading	6	6	75	12.5	2.5	80	5:1
August 17th 14 day reading	16	16	218	13.6	.37	89	36:1
August 24th 14 day reading	18	16	142	8.0	2.20	75	3.6:1
October 2nd 1st series 26 day reading	9	9	126	14.0	3.40	76	4:1
October 2nd ** 2nd series 26 day reading	10	10	71	7.7	1.60	79	4.8:1
Averages				6.9	1.4	72	

*One plant rooted since first reading
**Sterile sand, sterile pots, and disinfected plants used in this series of tests.

TABLE IV b

SUMMARY OF INOCULATIONS OF CLEAN RUNNER

PLANTS WITH CONIOTHYRIUM SP., SUMMER 1926.

Date of Inoculation	Total number of plants	Number of infected plants	Total number of lesions	Average lesions per inoculated plant	Average lesions per control plant	Percent infection due to inoculation	Ratio of increase in infection in inoculated plants
July 10 th, 1 st. series 27 day reading	24	13	65	2.7	.66	75%	4:1
July 10th, 2nd series 10 day reading	24	11	26	1.1	.32	71	3.4:1
July 10th, 2nd series 27 day reading	24	20	127	5.3	.66	87	8:1
July 21st, 1st series 15 day reading	31	5	13	.42	.05	89	8.4:1
July 21st, 1st series 41 day reading	31	31	560	18.0	5.3	70	3.4:1
July 21st, 2nd series 12 day reading	33	32	376	11.3	5.3	53	2:1
August 2nd, 12 day reading	10	10	143	14.3	0	100	---
August 24th, 14 day reading	18	14	145	8.0	2.2	72.5	4:1
October 2nd, 1st series 26 day reading	6	6	100	16.6	3.4	79	5:1
* October 2nd, 2nd series 26 day reading	10	10	117	11.7	1.6	86	7:1
Average				8.9	1.4	78.2	

* Sterile sand, sterile pots, and disinfected plants used in this series of tests.

TABLE IV c

SUMMARY OF CONTROL PLANTS

SUMMER 1926

Date of corresponding inoculations	Total number of plants	Number of infected plants	Total number of lesions	Average lesions per Control plant
July 10th 1st series 27 day reading	6	3	4	.66
July 10th 2nd series 12 day reading	25	66	8	.32
July 21st 13 day reading	39	2	2	.05
July 21st * 35 day reading	47	38	247	5.30
August 2nd 10 day reading	9	0	0	0
August 2nd 29 day reading	9	2	22	2.50
August 17th 14 day reading	29	5	11	.37
August 24th 14 day reading	27	12	62	2.20
October 2nd 1st series 26 day reading	7	7	24	3.40
October 2nd ** 2nd series 26 day reading	23	10	32	1.60
Average				1.40

* Eight plants rooted since first reading

** Sterile sand, sterile pots, and disinfected plants used in this series of tests.

The data compiled in columns 2, 3, and 4 are from actual records made in the field. Column 5 is obtained by dividing the total number of lesions by the total number of plants inoculated. Column 6 is obtained in like manner for the control series of the experiment. The seventh column shows the percent of infection in the inoculated plants of a series which properly is to be attributed to the inoculation. These percentages were obtained in each series by finding the difference between the average number of lesions per plant in the control and the average number of lesions per plant in the particular series of inoculated plants considered. This difference divided by the average lesions per plant for this particular series of inoculations gives the percentage of infection that may reasonably be attributed to the inoculation. Expression of the results in this way avoids the distortion which would arise if the infection was based on the number of plants showing a blackened or discolored area on the roots. A control plant showing one lesion would be given equal weight with an inoculated plant which might have 10 to 20 or more lesions.

Column 8 shows the increase in infection in the inoculated plants to that found in the control.

The lesions and rotted roots found and recorded on the inoculated plants were identical in appearance with the lesions and rotted roots found on plants which were growing in cultivated strawberry fields and on wild plants. These lesions at first are brown shriveled areas usually encircling the root and having white healthy root tissue adjoin-

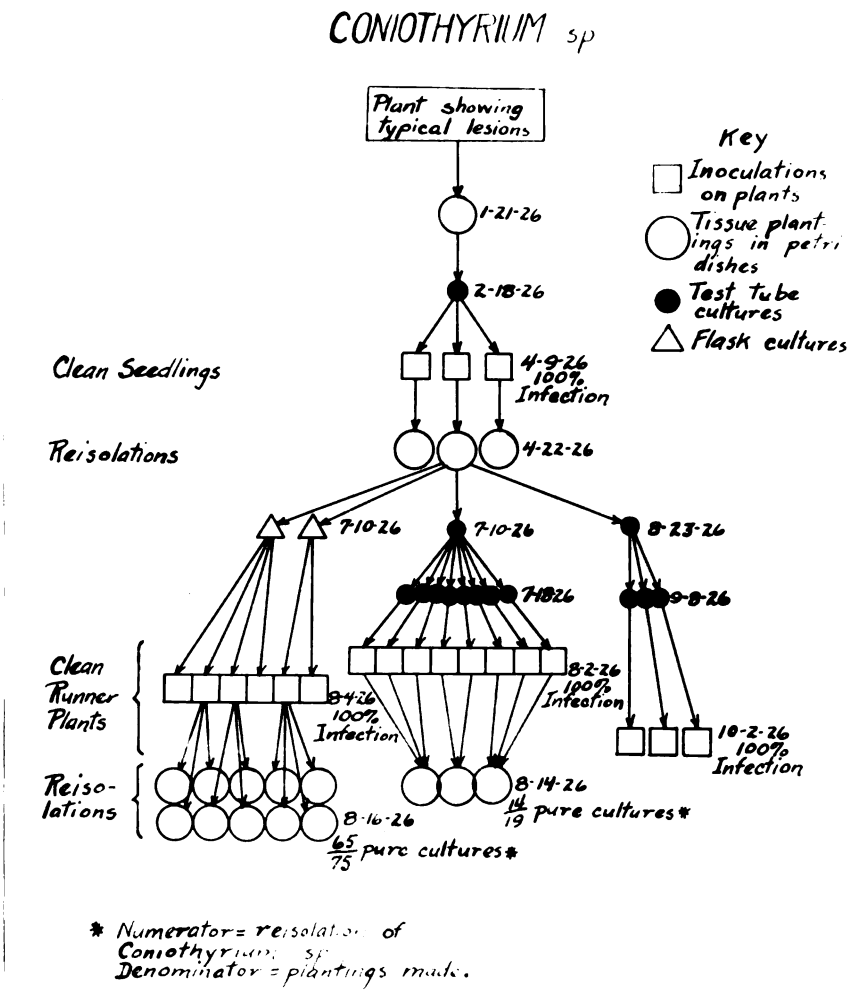
ing. These lesions were seen to increase in length until an entire root was involved.

Severe infection following inoculation with small pieces of mycelial mats of Coniothyrium sp. is shown in Figure 10. Mild infection following inoculation with a spore suspension of the same organism is shown in Figure 11. Severe infection following inoculation with Gloeosporium sp. is shown in Figure 12a, while early stages of infection are shown in Figure 13. Typical control plants are illustrated in Figures 12b and 14.

HISTORY OF CULTURES

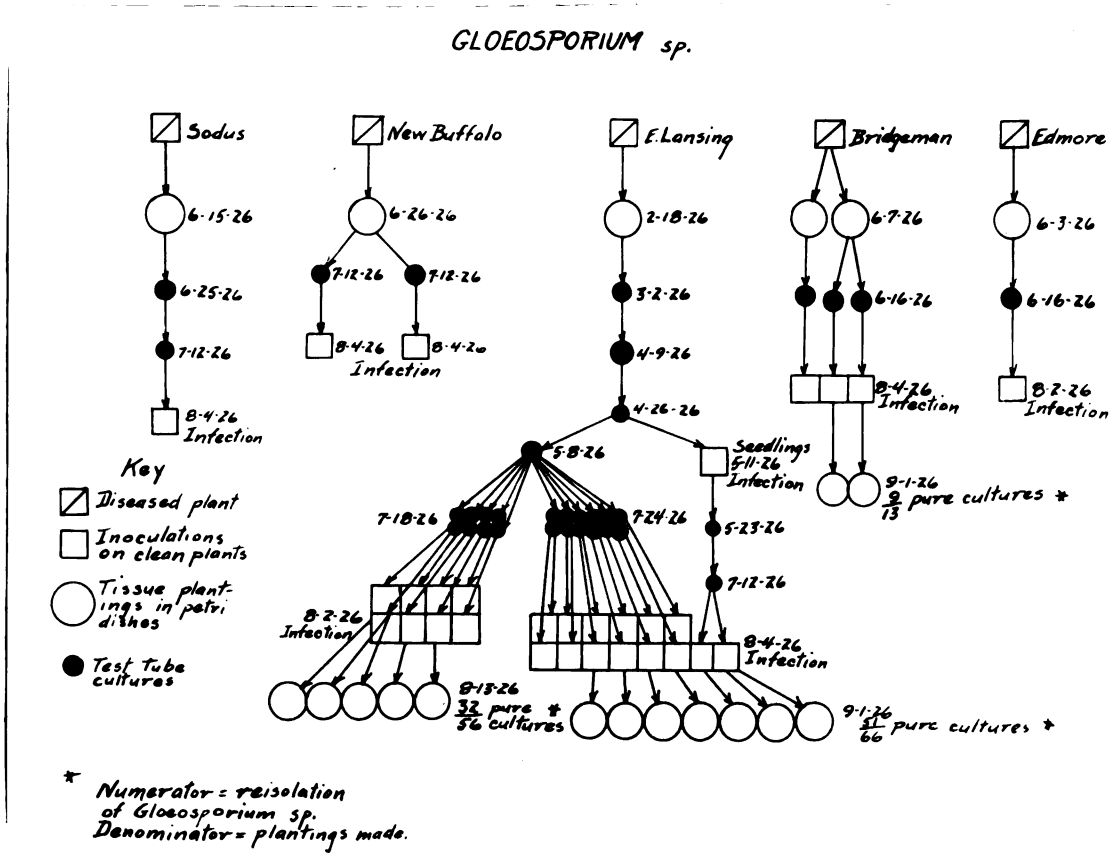
In making the inoculation tests upon runner plants in the field as further proof of the pathogenicity, part of the pure cultures used of the two organisms Coniothyrium sp. and Gloeosporium sp. were recovered from infected seedlings inoculated with these two organisms earlier in the season. In each case infection followed and typical black root disease lesions were demonstrated on the roots. The respective organisms were recovered in each case. Chart I gives a graphic representation of the history of one Coniothyrium culture. Similar histories could be shown of other cultures of this organism. Chart II shows similar representation of the histories of cultures of Gloeosporium sp. originating from different parts of the state.

CHART I



Graphic Representation of the Proof of Pathogenicity of Coniothyrium sp.

CHART II



Graphic Representation of the Proof of Pathogenicity of Gloeosporium sp.

ORGANISMS INVOLVED

The organisms which from the writer's work seem chiefly to be concerned in the black root disease of strawberries are two Fungi Imperfecti, as yet unidentified with any known species and possibly representing forms as yet undescribed. The following brief characterizations will suffice to record their salient characteristics.

CONIOTHYRIUM SP.

The pycnidia are ovoid to pyriform with a definite round ostiole, without prominent lips: black when filled with spores, but appearing dark brown after the spores are discharged. Walls thin almost membranaceous. Pycndia 160 microns to 300 microns in height and 140 microns to 170 microns in diameter.

Spores exuding in an inky black mucilagenous mass which accumulates at the apex of the pycnidium. Seen singly the spores are fuliginous one celled short ellipsoid to ovoid, rounded at both ends and usually 2-guttulate. Size 3.8--4.8 microns x 2.7--3.7 microns, average 4.2 x 3.1 microns.

This species does not appear to be the same as Coniothyrium Fragariae Oud. which is described as having somewhat larger spores, 11.6 x 9.3 microns.

GLOEOSPORIUM SP.

Acervuli on tissues of the strawberry plant 70--130 microns in diameter, cup shaped to saucer shaped when young but becoming flattened with age.

Spores produced singly on the ends of short conidiophores. As the mass of slime embedded spores increases the later conidiophores elongate to penetrate this mass to its surface so that eventually, when the spores are washed away, an old acervulus shows a mass of long slender conidiophores 40 to 50 microns long. Spores hyaline, one celled, slightly curved oblong, rounded at one end, slightly pointed at the other, 6.6--9.9 microns x 1.8--2.6 microns, average 8.6 x 2.2 microns. In mass the spores are a very pale brown. No distinct setae are present on the acervulus.

Note: When grown in culture the acervuli are often considerably larger than those described above, reaching a diameter of over 300 microns, but the spore size remains the same. The shape of acervulus and the fact that in cultures the basal tissues are rather thick and the production of the elongated conidiophores of the older acervuli are not typical of the commoner species of Gloeosporium but pending further cultural studies and studies in the field, it seems best for the present to designate it by this name.

TEMPERATURE RELATIONS OF THE TWO ORGANISMS

In order to find the optimum temperature for mycelial growth and spore formation a temperature apparatus was set up and arranged to run with nine compartments giving a gradual rise in temperature from 7 C. up to about 40 C.

A set of nine prune agar petri dishes was made having four cultures of Coniothyrium sp. per plate. These cultures were started from plantings of small bits of mycelium. A similar set of plates was made using Gloeosporium sp. Spore droplets were used in this case to inoculate the plates. The results of this experiment at the close of the seven days are shown in Table Va and Vb.

In this one series of experiments the optimum temperature for growth of mycelium was around 22--25 C. for the Coniothyrium with a maximum diameter of 4.5 cm. for colony and about the same for the Gloeosporium. Spore formation was limited to around 22 C. for the Coniothyrium while spore formation occurred freely over a wider range of temperature, 20.5--22.5 C., for Gloeosporium. These figures are tentative only and require further experimental work for verification.

ROOT TREATMENT EXPERIMENTS

Attempts were made to find a root treatment which would be effective in checking or completely controlling this disease. The first experiment involved the use of two chemical compounds in regular use as fungicides, viz: mercuric chloride and formaldehyde. Also two organic mercury compounds Semesan and Uspulan were used.

TABLE V a.

TEMPERATURE RELATIONS OF CONIOTHYRIUM SP.

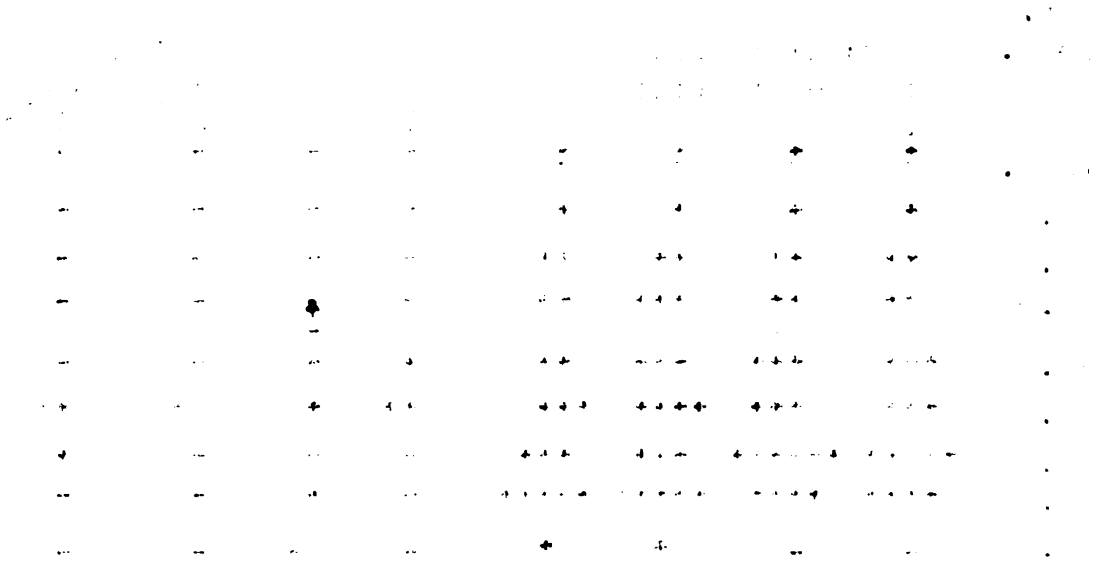
Av. Temp.	Mycelial growth				Spore formation			
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
6.0°C.	±	±	±	±	-	-	-	-
10.6	+	+	+	+	-	-	-	-
14.5	++	++	++	++	-	-	-	-
16.0	++	++	+++	++	-	+	+	-
20.5	+++	+++	+++	++	+	-	-	-
22.0	+++	+++	++++	+++	++	+	++	++
22.5	+++++	++++++	+++	+++	-	-	-	±
25.3	++++	++++	+++++	+++++	-	+	-	-
39.5	-	-	+	+	-	-	-	-

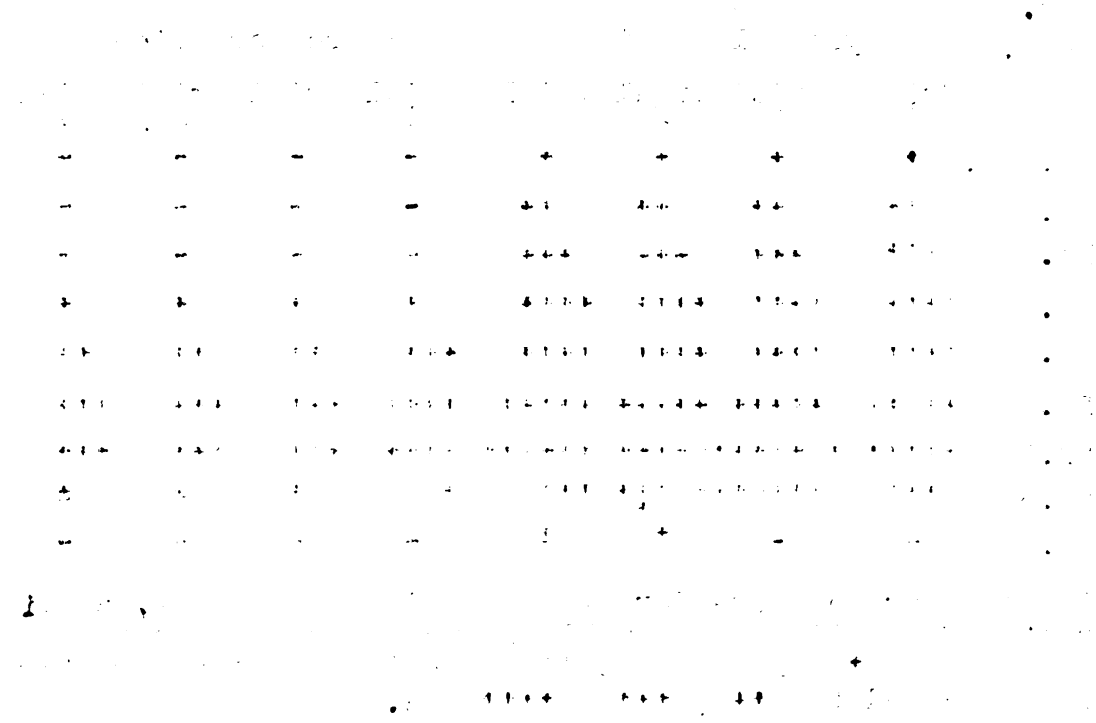
TABLE V b.

TEMPERATURE RELATIONS OF GLOEOSPORIUM SP.

Av. Temp.	Mycelial Growth				Spore Formation			
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
6.0°C.	+	+	+	+	-	-	-	-
10.6	++	++	++	++	-	-	-	-
14.5	+++	+++	+++	+++	-	-	-	-
16.0	++++	++++	++++	++++	+	+	+	+
20.5	++++	++++	++++	++++	+++	++	++	++
22.0	+++++	+++++	+++++	+++++	++++	+++	+++	+++
22.5	+++++	++++++	++++++	++++++	++++	+++	+++	+++
25.3	++++	+++++	+++++	+++	+	+	±	±
39.5	-	-	+	±	-	-	-	-

NO growth or no spore formation are indicated by -, a slight growth of mycelium or a small production of spores is indicated by +; greater mycelial growth or spore formation are indicated by ++ , +++ , ++++ etc.





These compounds were dissolved in water in various dilutions, and the plants plunged up to the crown in the solutions for periods of 30 minutes. These plants were then rinsed in tap water and set out in the experimental field. As controls every third row was planted with rows of untreated plants. Each treatment appeared six different times in the field nine rows apart. The Senator Dunlap variety of plants was used in four repetitions of the treatments while the Premier variety was used in two of the repetitions. Results do not warrant further details of the experiment. A condensed form of the results is given in Table VI. Three months after the plants were set sample plants were taken at random from all treated and untreated rows, and the root systems examined. Lesions and rotted roots were as abundant on the treated plants as on the untreated.

A second attempt to use a chemical compound to control this disease was undertaken. In this case copper sulphate was used. The purpose of this experiment was to endeavor to partially sterilize the soil to eliminate some or all fungi and yet not make the soil toxic to root growth.

Old rows of strawberries where killing was severe were selected, and the tops of the plants were hoed off even with the surface of the soil. The rows were then thoroughly dug and chopped up to a depth of 5 inches and a width of about 15 inches. These longer rows were then divided into seven short rows each 15 feet long. Copper sulphate solution was sprayed evenly over Plot #1 with a compressed air sprayer so that the plot received 10 gms. copper sulphate. Each succeeding plot

TABLE VI.

RESULTS OF ROOT TREATMENTS AS CONTROL FOR BLACK ROOT.

Row	Treatment	Dilution	Time	No. of plants		Growth read after 1 month	Disease read after 3 months
				Premier	Dunlap		
1	Control			100	200	stand excellent	many lesions on roots
2	$HgCl_2$	1/1000	15 min.	100	200	stand very poor	" " " "
3	HCHO	1/240	15 min.	100	200	" " "	" " " "
4	Control			100	200	stand excellent	" " " "
5	Semesan	1/400	30 min.	100	200	full stand	" " " "
6	Semesan	1/200	30 min.	100	200	full stand growth retarded	" " " "
7	Control			100	200	stand excellent	" " " "
8	Uspulan	1/400	30 min.	100	200	full stand	" " " "
9	Uspulan	1/200	30 min.	100	200	" "	" " " "
10	Control			100	200	stand excellent	" " " "

received an additional 10 gms. of copper sulphate up to the fifth plot where 50 gms. of copper sulphate was applied. The sixth and seventh plots were not treated. The plots were well watered after application of the copper solutions. The next day runner plants having an average of three 2--3 in. roots were set into these plots, 25 plants per plot.

The results of this experiment show little if any control of the disease infection. On inspection of the root system three weeks later, lesions were as prevalent on the plant roots from the treated plots as on those in the untreated plots. Furthermore the harmful effect of the copper solution was shown in the heavier treatments. In the 40 gm. per plot treatment the formation of lateral roots was inhibited while in the 50 gm. per plot treatment distinct injury of the larger roots was evident.

DISCUSSION

Evidence is presented of the parasitic nature of the black root disease of strawberry, and the proof is given for assigning the disease to two organisms, Coniothyrium sp. and Gloeosporium sp. The role that climatic factors play in augmenting the effects inaugurated by these organisms has been mentioned.

As has been shown, black root is to be found generally distributed in strawberry plantations in Michigan. The two organisms whose pathogenicity has been demonstrated have been isolated from strawberry roots gathered from many places more or less distant. The similarity of the lesions found on cultivated and wild strawberries

makes it seem possible that the wild plants also are affected with a black root disease of the same nature as that of the cultivated plants. This suggests that these fungi may be soil organisms of wide distribution. The fact that in this study two organisms have been found, each capable of producing typical black root conditions, together with the fact that the literature of the disease shows other organisms assignable as pathogens, makes it seem probable that the black root condition in strawberries may be the result of attack by any one of several pathogens perhaps naturally occurring in the soil.

The host range of the organisms isolated in this work is not known. It would be natural to expect them to attack plants closely related to the strawberry and probably other plants. No evidence of disease has been observed on plants of the genus Potentilla.

Although some work has been done on the control phase, the treatment of roots has not been successful as yet in checking the disease. If the organisms causing this disease are shown to be wide spread, root treatment will be useless. Whether these organisms and others which may cause the disease were originally spread by diseased plants which developed in nurseries cannot be said. This is possibly not the case, but it behooves the growers of plants for sale to furnish as strong, vigorous, and healthy plants as possible. The grower who sets plantations for the fruit crop must select only this kind of plants for setting. The practice of setting a plantation in the spring, harvesting the one crop the following year, and plowing up the plantation is one to be recommended. Another control measure to be recom-

mended is that of rotations. This is the setting of plantations on land where strawberries have not been grown for some years, or if possible where strawberries have never grown. The leading nurserymen practice this method to obtain as vigorous and as healthy plants as possible. The ultimate control is as with so many plant diseases, to be sought in the selection or breeding of disease resistant plants.

SUMMARY

1. Black root of strawberry was found from reports in the literature and collections, to be a widespread disease of cultivated and wild strawberries whose etiology had not been shown.

2. By inoculation with strawberry debris, the parasitic nature of the disease was determined and microscopic examination showed constant association of fungus hyphae in the lesions.

3. Coniothyrium sp. and Gloeosporium sp. have been isolated many times from typically diseased roots and their pathogenicity to plants grown under controlled conditions has been proved.

4. In several series of field inoculation tests using runner plants, the typical disease has been produced by each organism leaving no doubt of the etiological relations of these two forms.

5. Technical descriptions of these two species of organisms are given.

6. Treatment of strawberry plants with standard disinfectants before setting did not control black root.

7. General control measures such as would make for strong vigorous plant growth are recommended. Ultimate control of this disease will doubtless depend on selection of resistant strains.

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EXPLANATION OF PLATES

Plate I

Figure 1.
A 20 acre plantation near Edmore, Michigan, showing effects of black root.

Figure 2.
Plantation near Bridgeman, Mich. showing effects of black root.

Plate II

Figure 3.
Typical disease plant succumbing to black root.

Plate III

Figure 4.
Badly diseased plant from Edmore plantation showing lateral root production from blackened roots.

Plate IV

Figure 5.
Early stage of black root showing rotted roots and lesions from Edmore, Mich.

Plate V

Figures 6 and 7.
Typical lesions and rotted roots.

Plate VI

Figures 8 and 9.
Runner plants potted in clean sand, inoculated and controls.

Plate VII

Figure 10.
Severe infection following inoculation with Coniothyrium sp.

Plate VIII

Figure 11.
Infection following inoculation with spore suspension of Coniothyrium sp.

Plate IX

Figure 12a.
Severe infection following inoculation with Gloeosporium sp.

Figure 12b.
Control plant

Plate X

Figure 13.
Infection following inoculation with spore suspension of Gloeosporium sp.

Plate XI

Figure 14.
Control plant.

PLATE I

Figure 1.

Figure 2.

PLATE II

Figure 3.

PLATE III

Figure 4.

PLATE IV

Figure 5.

PLATE V

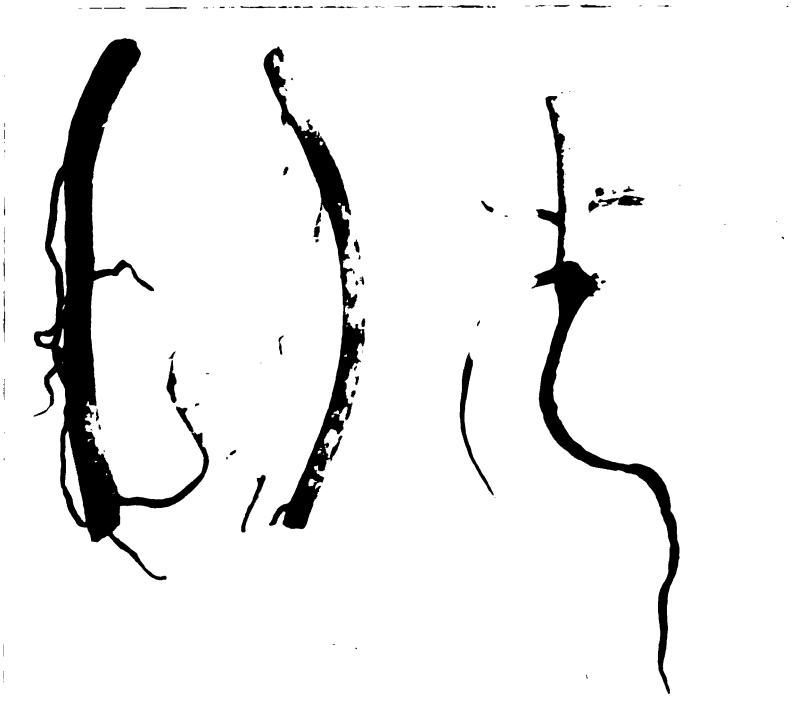

Figure 6.

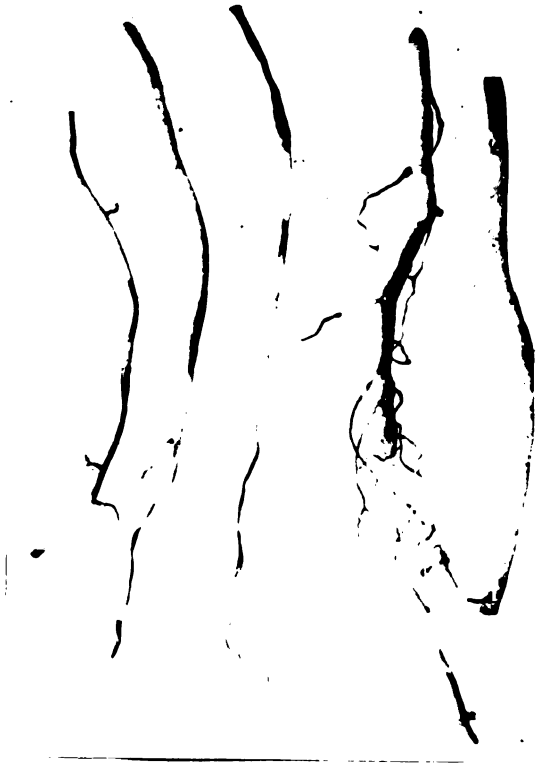

Figure 7.

PLATE VI

Figure 8.

Figure 9

PLATE VII

Figure 10.

PLATE VIII

Figure 11.

PLATE IX

Figure 12.

PLATE X

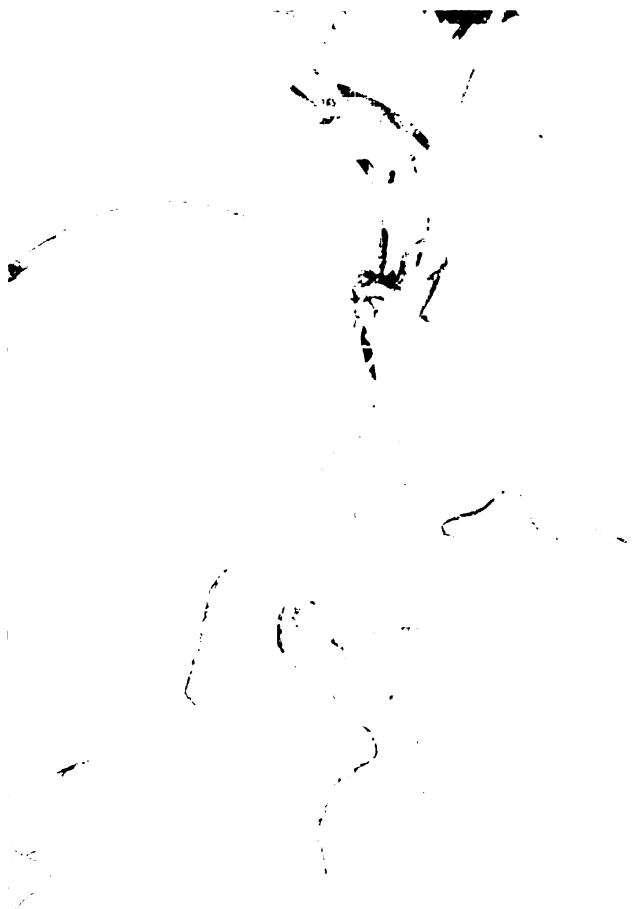

Figure 13.

PLATE XI

Figure 14.