FAUQUHER

A Study of the Manner of Infection of Corn by Diplodia Zeae (Schw.) Lév.

Botany

M. S.

1913

A STUDY OF THE MANNER OF INFECTION OF CORN BY DIPLODIA ZEAE (SCHW.) LÉV.

BY

SILAS EDGAR FAUQUHER

B. S. Earlham College

1909.

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN BOTANY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1913

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 6, 1913. 190

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Silas Edgar Fauquher B. S. Earlham College 1909.

ENTITLED A Study Of The Manner Of Infection Of Corn By Diplodia Zeae (Schw.) Lév.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master Of Science.

J. T. Barrett
In Charge of Major Work

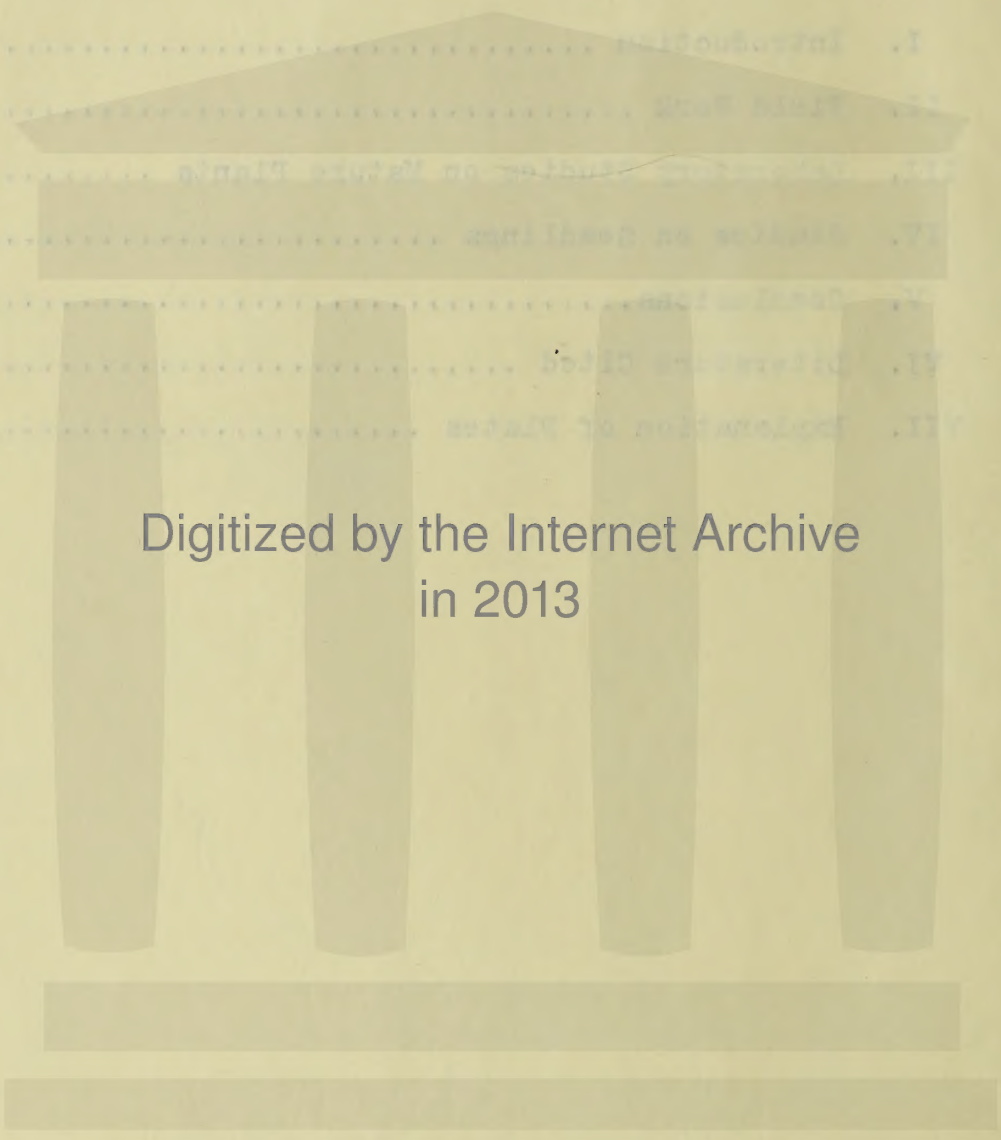
Chas. F. Hottes
acting
Head of Department

Recommendation concurred in:

Committee on Final Examination

CONTENTS.

-o-



I. Introduction.

Ear rots of corn have been especially studied at the Nebraska and at the Illinois experiment stations. Diplodia zeae is the fungus that causes what is probably by far the most serious of these rots. Burrill and Barrett (2,3) give the most complete account of its life history,which may be summarized as follows:-

The mycelium found on the ears is white and 2.5 to 4.0 microns in diameter (Plate I). It penetrates the tissue of the entire ear going from cell to cell extracting nourishment. As the ear becomes entirely involved or as the maturity of the host checks the growth of the organism pycnidia appear. The fruiting bodies on the husks are rather regular while those of the cob are quite irregular. (Plates II and III) The pycnidia contain central circular pores through which the pycnospores escape. These spores are brown,two-celled,and are borne on simple conidiophores. Paraphyses are present.

On the stalks the fungus appears in the fall as small dark specks under the rind. A great number of these were noted by the writer in the fall of 1912. Pycnidia continue to appear on the stalks until by the middle of May they are usually very abundant. In July,August,and September great numbers of spores are seen oozing out in black tendrils. The spores appear to be covered with a gelatinous substance and are capable of quick germination. On green actively growing stalks the fungus has not been observed,although the green shanks of ears are often badly infested. The pycnidia developed on the stalks are regular and usually occur most abundantly at the nodes. The mycel-

ium in the stalks is usually hyaline. The protoplasm is granular,sometimes vacuolated,and in the older stages filled with oil globules.

The earliest statement regarding the economic importance of the disease on corn,by Heald (4),does not deal with the question of infection. Barrett (1) states that infection is either early,resulting in a prematurely ripened and shriveled ear,of upright position,and with darkened husks,or later,rotting the ears to varying degrees,and with or without the above symptoms.

As a result of inoculation experiments Heald,Wilcox, and Pool (5) find that when the fungus mycelium is placed among the silks at the time of pollination,more typical Diplodia ears result than by any other method. This indicates to them the prevailing method of infection. Puncture inoculations into the husks at the time of pollination also yielded typical Diplodia ears. Puncture inoculations into the stalks yielded typical immersed pycnidia.

Burrill and Barrett (3) state that infection is by spores,and that under favorable conditions,as shown by their tests,large numbers of spores may be carried considerable distances by the wind. They found the fungus infecting only the shanks and ears of growing corn,however. Artificial inoculations of ears were found to yield typical Diplodia specimens.

Smith and Hedges (6) state that their examination of a bundle of maize plants indicated interior infection of the cobs,by way of the root system,instead of a local infection at the ears as thought by previous investigators. Mycelium was

found at many places in the interior of the stalks. They report on four pots in which seedlings were grown in soil inoculated with pure culture of the fungus. In one pot a stalk was found in which the mycelium extended up inside the stem two-thirds of its length. In other stalks the mycelium was found extending up shorter distances in the stems,and fruiting near the base. Mycelium was found in places in the roots,also. Experiments out of doors with inoculated soil were not completed. The authors conclude that infection is commonly from the soil into the roots,and from there up through the interior of the stem,and finally to the kernels to produce the ear rot.

The lack of agreement of the last mentioned investigation with those previous to it,and the fact that the previous workers had made no special study of the manner of infection led to the following work being undertaken,at the suggestion and under the direction of Dr.J.T.Barrett.

II. Field Work.

In the spring of 1912 an experimental plot on the University farm was prepared and planted with a high grade of white corn furnished by the agronomy department. The planting and inoculating of the various parts of the plot was done as follows:-

Pieces of internodes of corn stalks were thoroughly cleaned,placed in test tubes with water present,and sterilized in the autoclave. These were inoculated with pure cultures of Diplodia zeae and developed pycnidia in abundance. This material with the mature spores was used to place in the hills at planting. Corn meal in flasks,moistened with water and sterilized,was also inoculated and produced pycnidia in large numbers and was used in other hills in the same manner as was the stalk material. In addition stalks and ears of the previous season's growth,that bore pycnidia were broken up and used for planting in the hills. The plan of planting the plot is shown in Table I.

Row	First 24 hills,e.g. A1,A2,..... A24.	Next 12 hills	Last 13 hills
A	Inoc.with Diplodia on sterile stalks.	Check	Check
B	Inoc.with Diplodia on corn meal.	Check	Check
C	Inoc.with broken infected ears.	Check	Check
D	Inoc.with broken infected stalks.	Check	Check
E	Check	Check	Check

Table I.

This plot was cultivated by the station during the summer,and early in September while the corn was still green and growing it was visited by the writerand a second series of artificial inoculations made. All except a few late inoculations were made in the east quarter of the plot,i.e. in the last twelve hills of each row at the farthest distance from the first inoculated part. In this work spores from pure cultures were used. In some these spores were placed among the silks or at the base of ears. In other specimens spores were inserted in incisions in the stalks,at both nodes and internodes. These inoculations were made rather late in the season,September 9, and few typical Diplodia ears developed. Enough infection resulted however to confirm the previously established fact of successful artificial inoculation. No special work was done with these inoculated ears and stalks,but a few of the ears are reported in the succeeding discussion.

Close observation of all ears in the plot was made September 23,and from this time on through the fall. When first inspected the ears were getting mature and seven stalks bearing typical Diplodia ears were found. Six of these were in the section of the plot inoculated in the spring and the other was in the east end. Using row numbers as in Table I,and numbering from 1 to 49,1 to 24 being the spring inoculated in all the rows except E which is all a check,the location of the hills bearing Diplodia ears is shown in Table II.

A8 - one ear - inoculated part of the plot

A12 - " " " " " " "

A14 - " " " " " " "

A19 - " " " " " " "

B20 - " " " " " " "

C18 - " " " " " " "

B48 - " " - non-inoculated part of the plot

Table II.

As mentioned above a search was kept up for Diplodia ears that developed later than September 23, all the corn being left for husking until November 1, or later. Ears that were infected with Diplodia were recorded if they showed pycnidia. If not, and there was a suspicion that they might have the disease in a somewhat earlier stage, they were left for further development. On a few the causal organism was not definitely identified until several days later. The number of ears developing the disease later, together with a summary of the earlier ears given in Table II, coupled with a total of the ears in the plot is shown in Table III.

Row	Ears with Diplodia Sept. 23	Diplodia later	Total ears
A	A8, A12, A14, A19	A7	136
B	B20, B48	B26, B39, B46	132
C	C18	C6	118
D	None	None	131
E	None	E3, E7, E17, E36(2), E37, E42	146
Total	7 ears by Sept. 23	12 ears later	663

Table III.

This shows that finally nineteen ears, or 2.8% of the ears in the plot, became infected with Diplodia. Eight ears, 1.2%, occurred in the section inoculated in the spring. E42 was not due to the artificial inoculation in the stalk, nor was B48, in those taken September 23. Six, 0.9%, occurred in the check. Five, 0.7%, resulted from fall inoculations in the ear. It is clear that the checks produce Diplodia ears to practically the same extent as do the stalks in the inoculated hills. As has been well established by Burrill and Barrett (3), spores are carried a much greater distance than that involved here. It would appear that the infection here is due to the blowing of spores from inoculated hills to the ears.

In the period from November 8 to 20, when the stalks were all dead, an examination was made to determine upon how many stalks Diplodia could be found growing saprophytically, as indicated by the presence of pycnidia on the outer surface. The results in the plot are shown in Table IV.

Row	Inoculated or check?	Stalks and hills showing Diplodia	% of hills
A	24 inoculated hills	33 stalks in 18 hills	75
A	25 check hills	29 stalks in 19 hills	76
B	24 inoculated hills	42 stalks in 22 hills	91
B	25 check hills	26 stalks in 16 hills	64
C	24 inoculated hills	28 stalks in 16 hills	66
C	25 check hills	26 stalks in 19 hills	76
D	24 inoculated hills	32 stalks in 18 hills	75
D	25 check hills	30 stalks in 16 hills	64
E	49 check hills	35 stalks in 27 hills	55
	Average % of hills showing Diplodia -------		69.7

Table IV.

A count similar to the preceding was made on two plots near the experimental one. In a row of the same length Diplodia was found fruiting on 46 stalks,in one of these plots. In the other,which was farther away from the experimental plot, Diplodia was found on 7 stalks in 7 different hills.

Table IV shows that in over two-thirds of all the hills of the experimental plot,where inoculated material was present in some of the hills,the fungus very rapidly took hold of mature stalks. Only those showing pycnidia on the surface are recorded,and it must be remembered that many other stalks were evidently infected with the pycnidial stage not yet present. These observations together with those on the other two plots show the quick saprophytic growth on the stalk.

III. Laboratory Studies on Mature Plants.

It was now desirable to make a more intensive study of stalks, especially of those bearing diseased ears. A collection of fifty stalks with typical Diplodia ears was gathered in a field about a mile distant from the University farm. Laboratory tests were made with corn plants from the experimental plot and with typical infected stalks from the outside field.

Two kinds of culture media were at first made use of. Glucose potato agar was made by cooking a potato of about 250 grams in 1000 cubic centimeters of water and adding 20 grams of 2% glucose and 30 grams of agar and making up to one liter. This gave a material upon which Diplodia fruited but not quickly. The other medium was corn meal agar, made by cooking four teaspoonsful of corn meal, adding 10 grams of agar, and making up to one liter. This was about the same in results as the potato agar. It was found by trial that a considerably more concentrated corn meal agar, using 80 to 100 grams of the meal to the liter, gave a medium that produced more rapid fruiting of the fungus, pycnidia appearing in about one week. Checks were run constantly with all the media, thus insuring that all would produce pycnidia.

The presence or absence of the fungus was determined by cultural methods. The cleaned stalks were flamed lightly, and with the aid of sterile instruments pieces of the tissue were removed from the pith at varying depths beneath the rind. Usually pieces were removed from each node and internode from near the surface of the ground up to one or two nodes above the ear. In the cases of contaminations or of inconclusive results

duplicates were run. These transfers of tissue were made to agar slants in test tubes and there left to develope. In instances where Diplodia was present,as shown by numerous checks, the growth in the tubes was soon evident. The color and characteristic appearance of the mycelium usually indicated the presence of Diplodia,but in all cases the cultures were allowed to progress to the formation of pycnidia and spores before definite data was taken. Then the presence of the fungus was recorded. This result appears in Table V. From the seven stalks of Table II 186 transfers of tissue were made to as many tubes. Besides these seven stalks (those bearing typical Diplodia ears by September 23) six of the stalks later found bearing diseased ears were examined in a similar manner. These are reported in the second division of Table V. Then two stalks showing no Diplodia ears,but growing in the west end of row A where the hills were inoculated in the spring,were examined. They are recorded in the third division of the table. Finally,five typical stalks from the outside field were used and the results are reported in the fourth division of the table.

The table shows from what hills the stalks were taken and when. The results of examination are recorded beginning with the first node above the ear (1N) and from there on down, including the shank,to the eighth node below the ear (8N). This takes the stalks down to about the surface of the ground.

Hill No.	Date taken	1N	1I	Shank	1N	1I	2N	2I	3N	3I	4N	4I	5N	5I	6N	6I	7N	7I	11 8N
A8	Sept.23	-	-	D	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
A12	"	x	x	-	D	D	D	D	x	x	x	x	x	x	x	x	D		
A14	"	-	-	-	x	x	x	x	x	x	x	x	x	D	x	x	x	x	x
A19	"	-	-	D	D	x	x	x	x	x	x	x	x	x	x	x	x		
B20	"	-	-	-	D	D	x	x	x	x	x	D	D	D	D	D	D	D	x
B48	"	-	-	-	D	x	x	x	x	x	x	x	x	x	x	x	x		
C18	"	x	D	-	D	x	x	x	x	x	x	D	D	x	x	x	x		
A42	Oct.11	x	x	x	D	D	x	x	-	-	-	-	-	-	-	-	-		
B26	Nov.4	-	-	D	D	D	x	x	x	-	x	-	x	-	-	-	-		
B39	"	x	x	-	x	x	-	x	-	x	-	-	-	-	-	-	-		
B46	"	-	x	-	D	x	-	x	-	x	-	-	-	-	-	-	-		
C6	"	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x		
E42	Oct.11	-	x	D	-	x	x	x	x	x	x	x	x	x	-	-	-		
A12'	Oct.15	-	-	-	-	-	-	-	-	-	-	-	x	x	x	x	x	x	
A15	"	-	-	-	-	x	-	x	-	x	-	x	-	D	x	x	D	D	D
G11	Sept.30	x	x	-	D	x	x	x	-	-	-	-	-	-	-	-	-		
G12	"	x	D	-	x	x	x	x	x	-	-	-	-	-	-	-	-		
G13	"	x	x	-	D	x	D	x	x	x	x	x	-	-	-	-	-		
G14	"	D	x	-	D	D	x	x	-	-	-	-	-	-	-	-	-		
G15	"	x	D	-	x	x	x	x	-	-	-	x	D	x	-	-	-		

Legend:- D -- found Diplodia ; x --transfer of tissue and found no Diplodia ; - -- indicates no transfer of tissue was made.

Table V.

The most important conclusion from the results tabulated in Table V is evident at a glance. In no stalk is the fungus found all the way up to the ear. As the vitality of the stalk decreases, thus furnishing more suitable conditions for saprophytic development of the fungus, its rapidity of growth soon becomes evident. Thus in five of these stalks the fungus was found at various places around the bases of the stalks, and in some instances it had progressed a short distance upwards. It never went up to the ear, as was shown by repeated cultural tests with tissue from the central portions of the stalks. In order to supplement this data some tests were made late in the spring of 1913. Stalks having Diplodia ears were used. In all except one instance (a stalk from the outside field, G3) the same stalks that are reported on in Table V were taken. They were thoroughly cleaned and then flamed lightly. With a sterile knife an entire cross-section was removed from near the center of each stalk, that is about midway from the ground to the ear. These cross-sections were placed in petri plates containing concentrated corn meal agar. At the same time checks were run with parts of these same stalks where the mycelium was known to be present to see that the mycelium was alive and would grow on the particular medium used. In these checks Diplodia growths with production of pycnidia were found. Therefore the results of the transfers of cross-sections may be noted with interest. This report as given in Table VI has the node or internode from which the cross-section was taken numbered from the ear down.

Hill No.	Where cross-section was taken	Results
A8	4th internode	Diplodia found
A19	5th node	None found
B39	2nd internode	None found
B48	4th internode	None found
C6	4th internode	None found
G14	2nd internode	None found
G15	4th internode	Diplodia found
G3	3rd node	Diplodia found

Table VI.

The saprophytic growth might reasonably be expected to carry the mycelium the length of the stalk by this time, as these transfers of cross-sections of stalks were made in March. However, it is seen that in only three of the eight cases was it found. In G3 no previous tests had been run, it being as stated above another of the stalks from the outside field. In G15 a previous test (Table V) had revealed Diplodia just below the place of transfer. Putting together the evidence from Tables V and VI it is clear that the central portions of these stalks did not contain the mycelium, and that in no instance could the infection have been from the soil. The ordinary infection appears to be at the ears and from there the mycelium may travel a short distance in either direction. The finding of mycelium several inches from the infected ears can be ascribed to the later saprophytic growth of the fungus. The transfers of tissue were made from two to several weeks after cutting the stalks. Again, as is shown in Table IV, the fungus very rapidly appears on the bases of matured stalks. Although at this time

there may be some infection from the matured roots,it appears that mainly the spores find lodgment on bases of stalks,and principally at the nodes. An early production of pycnidia results. This should be borne in mind in noting such stalks as the one from hill B20 in Table V. Here at seven places,from the surface of the ground up to the fourth internode below the ear,or about one third the distance up to the ear,mycelium was found in the stalk. This is evidently due to the saprophytic growth of the mycelium.

IV. Studies on Seedlings.

It was now desired to study the problem of infection and growth with young corn plants, and thus to supplement the observations made above. For this purpose a series of pot cultures was started during the latter part of February, 1913. For this work six and eight inch pots were used mainly. After being dipped in a copper sulphate solution these were filled with soil. Some was steamed and some ordinary soil. In one half the pots the corn used was white, and in the other half yellow. The material used for inoculation was pure cultures of the fungus grown either on corn meal or on corn meal agar. The positive germinating power of the spores was ascertained in each instance. Some of the seed was planted dry, some soaked, and some germinated. Part of the seed was soaked for from five to ten minutes in a 0.16% solution of formalin. Preliminary tests showed that this strength formalin did not hinder germination. It was used in an effort to kill chance spores of organisms that might be lodged on the outside of the seed corn. Table VII shows how the pots were planted. Those marked "treated" are understood to have been subjected to the formalin treatment. Each rectangle in the table represents one of the 36 pots, and there is given the details not only of planting but of all inoculations, not only at planting but also later. It is understood that the first two rows lettered C are checks. Some pots from this section were used later in the season for artificial inoculation as is indicated in C1, C2, C7, and C8. The pots numbered X and Y are large twelve-inch ones.

Yellow	C6 Dry untreated	C7 Soaked treated Inoc.Apr.14 in slits in stalks	C8 Germinated treated Inoc.Apr.4 in slits or leaf sheath	C9 Soaked untreated	C10 Germinated treated
White	C1 Dry untreated Inoc.Apr. 22 in tissue macerated	C2 Soaked treated Inoc.Apr.15 slits in the stalks	C3 same as C8	C4 same as C9	C5 same as C10
Yellow	A1 Dry untreated Inoc.at planting	A2 Soaked treated Inoc.at planting	A3 Germinated treated Inoc.at planting	A4 Germinated, treated. Inoc. at 2 in.	A5 Germinated treated
White	B1 same as A1	B2 same as A2	B3 same as A3	B4 same as A4	B5 same as A5
Yellow	D1 Dry untreated Inoc.at planting	D2 Germinated, treated Inoc.at planting	D3 Germinated treated Inoc.infected stalks	D4 Soaked untreated Inoc. at planting	D5 Germinated treated
White	E1 same as D1	E2 same as D2	E3 same as D3	E4 same as D4	E5 same as D5
F1 and F2 Yellow	X WHITE Germinated, treated		Y YELLOW Germinated treated	F1 Germinated treated	F2 Germinated treated Inoc.at 2 in.
G1 and G2 White	Inoc.in leaf sheath Apr.14		Inoc.in macerated areas or slits on Apr.15	G1 Germinated treated Inoc.at 4-6 in.	G2 same as F2

C9, C5, D1, D2, E1, E2, X, F1, F2, G1, G2 are unsteamed soil; all others contain steamed soil.

Table VII.

Until March 11 nothing was done with the seedlings, except to make some early inoculations of young plants as in-

dicated in TableVII. All artificial inoculations made on the seedlings were with pure cultures of the fungus. The pots, after the inoculations,were covered with bell jars for twenty four hours to prevent drying of the plants and to give the best opportunity for infection. It is a well known fact that the germination of seed and early growth of plants in steamed soil is slower than in ordinary soil. This was noticeable in the series. Later little difference could be seen,and throughout the experiments the gross appearance of all the growing stalks was practically the same. There was no external evidence at any time of the plants being diseased.

The first examination of seedlings was made on March 11. From this time on until the last of May examinations of plants taken from the pots were made at frequent intervals. A gross inspection was made as to the general appearance of the growing stalk and the root system when the plant was taken up. A microscopic search was made in the tissues of the stem and of the root for mycelium or spores of Diplodia,or for any abnormal condition that might indicate disease. Finally a series of agar plates was run. Transfers of tissue from various parts of the stem and root were made to the plates,and subsequent growths carefully examined.

The medium used was the concentrated corn meal agar. A drop of lactic acid was added to each plate to prevent bacterial contaminations. Tests made with Diplodia on this medium showed that the fungus would fruit quickly and abundantly in the plates.

The method of handling and examining the material was

as follows:-

The seedlings were first washed and then put either into a solution of mercury bichloride or into boiling water. A 0.1% solution of the bichloride was used and the plants left in it for from three to five minutes. Where hot water was used the plants were dipped into boiling distilled water for three seconds. Almost all the seedlings were treated in an attempt to kill spores that are always encountered on the outside of material of this nature exposed to the air. Contaminations were thus materially reduced and the corn tissue was not injured. Examinations of tissue after exposure to the treatments for longer periods than used in the tests showed that the cells were not plasmolyzed. That all fungi were not killed was evident from the abundant growth of Aspergillus,Penicillium,and Fusarium in some of the plates. Spores of Diplodia inserted just beneath the thin leaf sheath in stalks were not injured by either of the treatments above,and transfers to plates produced pycnidia. A few of the larger stalks where later artificial inoculations had been made simply had the outer leaf sheath removed and direct transfers of cross-sections of the stem made thus.

Transfers of cross-sections of stem or root or both were made from 162 different plants. Of this number 104 had been inoculated at the time of planting. In this lot Diplodia was found absent in root and stem,by all the tests above. One exception should be noted. In a transfer made to a plate early in the work Diplodia developed. This was from a piece of root grown in pot B3,where the seed had been inoculated with spores. The plant was evidently not so well cleaned but that a stray

spore was left clinging to the tissue and germinated and grew in the agar plate. From a great many of the plants transfers were made of cross-sections from two or three places in stem and root.

From the check pots 23 plants were examined. In gross appearance these were like the inoculated plants. No Diplodia developed from any of these.

From those artificially inoculated after the appearance of the plant 35 were examined. These had had solutions of spores applied to the young plants or had been inoculated by the insertion of spores into incisions or into macerations in the stalks. From these 35 plants 5 developed Diplodia in plates and this was not unexpected. In many instances the microscope revealed spores in the tissue. It was practically impossible to be sure that the cross-sections of the stem just above or below the point of inoculation did not contain some spores. A close observation of the seedlings revealed spores around the place of inoculation in the majority of instances. These were ungerminated in the main. Spores lying at the ragged edge of an incision or in macerated tissue were often found germinated. This seems to be due to their opportunity here to grow saprophytically, or at least semi-saprophytically. Where the spores were seen on green, actively growing tissue they were not germinated. This was the uniform observation and it should be said that even in the deadened macerated tissue only a small percentage of spores were germinated. These same spores when put in a 0.1% glucose solution germinated readily in about twenty four hours. (Plate IV) Figures 1 and 2 show a typical example. In

1 are seen spores lying on the green growing tissue of a stalk where an incision had been made and spores inserted twenty four days previously. A great number of spores were seen in the field of the microscope and none were germinated. The figure is a camera lucida drawing of a small part of the mount. This mount was covered with a 1.0% solution of glucose and left for twenty four hours. The result is shown in Figure 2 where a large percentage of the spores have germinated. This shows clearly that the green,actively growing tissue is not a suitable medium for the germination and growth of the spores.

V. Conclusions.

The preceding studies lead to but one main conclusion. the stalks of a large number of mature corn plants and both the stalks and roots of a large number of plants in early stages of development have been examined. In none of these is the mycelium found in the roots. There seems to be no evidence to indicate an infection other than that from the blowing of spores to the ears,and thus a local infection. Practically every field condition has been imitated and the results appear to be conclusive.

The evidence shows that the fungus is capable of making more rapid progress when growing saprophytically than when living as a parasite,especially in stalks. With this fact in mind it seems that a different interpretation should be made of the observations of Smith and Hedges (6) as regards their finding of mycelium in the stalks sent in for their examination. Instead of indicating an infection from the roots up through the stem,the presence of mycelium in the stem is evidently due to its saprophytic growth and spreading in the dead tissue. The growth on green stalks occurs but it is limited as compared with the growth on dead stalks. The growth appears to be a semi-saprophytic one,comparable to that found in some of the macerated tissue inoculations of plants in the pot cultures as described above. Apparently the growth on the almost mature stalks is also a semi-saprophytic one. The maximum growth seems to be in dead tissue and runs down to a minimum in the maturing stalks. In the young stalks,e.g. those of a foot in height, the investigations show no infection whatever. The writer be-

lieves that no hard and fast line can be drawn as to the time when the corn tissues become susceptible to the inroads of Diplodia,but that the dangerous time is at the time development is about complete. This prevents,from a lack of time,the fungus' traveling up inside the stem to infect the ear. Again,when the spores are blown upon the developing ear there is a time,comparatively brief,when they can infect. It seems that infection is considerably more difficult either before or after this time.

It would appear that the observations of all the previous workers have been true as regards the presence of the fungus,but that the interpretations of these observations as bearing on the manner of ear infection have been to some extent at least incorrect.

VI. Literature Cited.

1. Barrett,J.T. Dry rot of corn and its causes. Science,N.Ser.XXVII,pp.212-213. 1908.

2. Burrill,T.J. and Barrett,J.T. Dry rot of corn. Circ.117,Ill. Exp. Sta. 1908.

3. _____ Ear rots of corn. Bull. 133,Ill. Exp. Sta. 1909.

4. Heald,F.D. New and little known plant diseases. Science,N.Ser. XXIII,p.624. 1906.

5. Heald,F.D.,Wilcox,E.M.,and Pool,V.W. The life history and parasitism of Diplodia zeae (Schw.) Lev. Twenty secondth Ann. Rept. Nebr. Exp. Sta. 1909.

6. Smith,E.F. and Hedges,Florence. Diplodia disease of maize. Science,N.Ser. XXX,pp.60-61. 1909.

VII. Explanation of Plates.

Plate I.

Fig. 1. An ear infected with Diplodia at the base.

Fig. 2. An ear entirely involved with Diplodia.

Plate II.

Fig. 1. A stalk bearing two typical Diplodia ears. Note pycnidia around the base of each ear.

Plate III.

Fig. 1. Three shanks of ears bearing typical pycnidia. (The two larger ones in this photograph are from Bull. 133, Ill. Exp. Sta. 1909.)

Plate IV.

Fig. 1. Spores from the green stem of a seedling. Had been inoculated 24 days previously in an incision in the stalk. With aid of camera lucida.

Fig. 2. Spores of same mount after being left for 24 hours in 1.0% glucose solution. Note large percentage germinated. With aid of camera lucida.

Fig. 1 Fig. 2

Fig. 1

Fig. 1

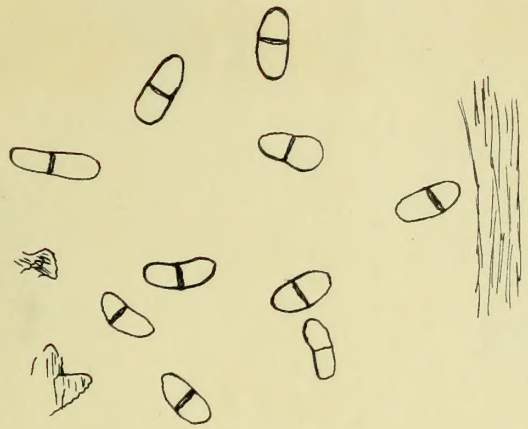


Fig. 1

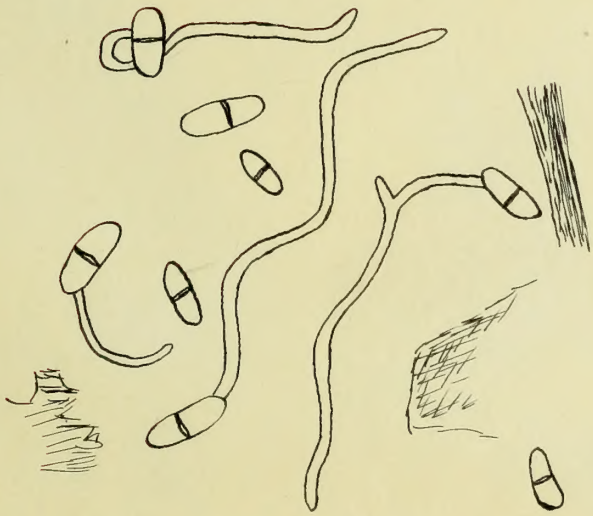


Fig. 2