THESIS

MODE OF ENTRANCE OF FUSARIUM INTO WHEAT SEEDLINGS

FRANCIS O. HOLMES

DEPARTMENT OF BIOLOGY AND PUBLIC HEALTH
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction: The Disease</td>
<td>1</td>
</tr>
<tr>
<td>Object of investigation</td>
<td>6</td>
</tr>
<tr>
<td>Former Work</td>
<td>9</td>
</tr>
<tr>
<td>Methods:</td>
<td>11</td>
</tr>
<tr>
<td>Seedlings</td>
<td>11</td>
</tr>
<tr>
<td>Inoculating</td>
<td>15</td>
</tr>
<tr>
<td>Killing and embedding</td>
<td>15</td>
</tr>
<tr>
<td>Staining and mounting</td>
<td>18</td>
</tr>
<tr>
<td>Experiments</td>
<td>20</td>
</tr>
<tr>
<td>Fusarium sp. Strain I</td>
<td>21</td>
</tr>
<tr>
<td>Fusarium sp. Strain II</td>
<td>26</td>
</tr>
<tr>
<td>Fusarium sp. Strain III</td>
<td>33</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>35</td>
</tr>
<tr>
<td>Helminthosporium sp.</td>
<td>53</td>
</tr>
<tr>
<td>Discussion of results</td>
<td>56</td>
</tr>
<tr>
<td>Summary of results</td>
<td>60</td>
</tr>
<tr>
<td>Bibliography</td>
<td>61</td>
</tr>
</tbody>
</table>
INTRODUCTION

Fusarium-blight of the cereal crops is a fairly wide-spread disease of wheat, barley, oats, rye and many grasses of the United States. It attacks both the root systems, causing footrot, and the stalks and leaves, causing wilt.

The causal organism is Gibberella saubinettii, (Mont.) Sacc., a fungus closely related to the large genus Fusarium. This species has a number of other names which will be found in the list of synonyms on page 4.

The disease is prevalent throughout the eastern states and in the central wheat producing area of the United States. In Europe it occurs in England, France, Italy, Germany, Austria, Holland, Denmark, Norway, Sweden and Russia. It is also reported from Siberia and Australia.

The loss of wheat from this disease in the states reporting in 1917 was over ten million bushels according to the Plant Disease Survey (1). The total

(1)
loss in all states was probably nearly twenty million bushels.

The crops affected have a lower percentage of germination, an increased death rate among young seedlings, wilting of full grown plants, and final blighting of the heads containing the seeds.

The kernels which are blighted may be very much smaller than normal, light in weight, and unable to germinate. This condition obtains if the infection is early in the development of the seed. Later infection causes but slight shrinking, slight decrease in weight, with low percentage germination. Infection just before ripening causes almost no change in the appearance of the seed, except that light pink spots may be observed. Such seeds usually germinate, but wilt when still young plants as a result of infection from the seed. The pink spots on the infected seeds contain conidia characteristic of the fungus, being found as well on other parts of diseased plants at times.
The conidia are typically, sometimes all, 5-septate, measuring 45µ to 65µ by 4.2 to 5.5µ; 3-septate forms measure 35 to 45µ by 5 to 5.5µ; seldom 4-septate; rarely 6-, 7-, or more septate, 60-75µ by 4 to 5µ. (1)
LIST OF SYNONYMS.

The proper name of the organism concerned in the blight of wheat is Gibberella saubinettii (Mont.) Sacc.

Synonyms as given by Atanasoff (1):
Gibbera plicaridis (Fr.) f. zeae maydis, Rehm: Ascomyceten 381. From New Jersey, 8, 1875, J. B. Ellis.
Fusarium roseum, Autorum.

(4)
Fusarium tropicalis, Rehm, 1898, in Hedwigia, Bd. 37, p. 194.

Giberella tritici, P. Henn. 1902, in Hedwigia, Bd. 41, p. 301.

OBJECT

The object of this thesis investigation was to discover the mode of entrance of the fungus causing wheat blight into the wheat seedling. Previous workers have observed the death of the seedling after inoculation, or leaf-spot after spores or mycelium have been placed on the leaf. "Conidia, ascospores, and mycelium of the organisms, when placed on normal young plants, with or without wounding, cause infection." (1) It has also been noted that "these organisms can invade the tissues of the seed, straw, and heads of the cereal crops after ripening and harvesting if the conditions are favorable." (1) More particularly Atanasoff states "the coleorhiza and coleoptile, which always die shortly after the formation of the permanent roots and the appearance of the first foliage leaf, seem to offer a good medium for the establishment of the various species of Fusarium, which then penetrate into the tissues of the permanent roots and the first foliage leaf,
causing rotting and browning of the invaded portions."

(1) No histological evidence has been found for this statement, and the work reported in this paper was an attempt to obtain such a condition and to discover the exact manner in which the fungus is able to penetrate the tissues, whether into the cells, between the cells, through stomata of the first leaf, or in some other way.

It must be pointed out that there are possibilities for mistakes in the macroscopic examination of infected seedlings. Even after careful surface sterilization, seeds are almost sure to contain some fungus, in many cases the organism of this disease; inoculation without apparent wounding may cause "leaf-spot" or browning on account of osmotic conditions at the point where the fungus is deposited. So that an investigator may place spores on the surface of a leaf, observe browning of the point of supposed infection, wilt of the entire plant, browning of the roots and all the symptoms of Fusarium seedling
blight without any real action of the infective material. It is only by examining sections through the point of inoculation that it can be shown whether or not the organisms used were effective.
former work along similar lines

Histological investigations similar to the one reported in this paper have been carried on for many of the fungus diseases of plants.

In 1886 DeBary discovered that a species of Botrytis entered the leaves of broad beans after forming appresoria, under which the tissue softened and blackened.

Two years later, Ward described a lily disease in which the hyphae exuded a viscous fluid, attaching the threads to the surface and softening the cellulose walls to aid the entrance of the fungus.

Busgen showed in 1893 that Botrytis cinerea formed appresoria and then infection threads, which passed through the walls of the cells after these had been softened by enzyme activity.

Dey in 1919 found that Colletotrichum lindemuthianum entered by means of a peg-like infection hypha near which there was some enzyme action after pene-
A number of disease producing organisms also pass through the stomata of the leaves, or send only haustoria into the cells for nourishment.
METHODS

(1) Seedlings:

Seedlings were grown from seed surface-sterilized as follows:

- 50% alcohol . . . . 30 seconds
- Water . . . . . . . 30 minutes
- 2% HgCl₂ in 50% alc. 2 minutes
- Wash thoroughly in sterile water

The seeds were then grown on agar plates until the young shoot was about one half centimeter in length.
The sketch on the next page shows an enlarged seedling at the proper stage for inoculation. The appearance of a number of the seedlings on a carefully prepared agar plate is also shown. The seeds should be arranged with a sterile needle so that the first leaf will develop in a normal position to allow easy and accurate inoculation.

Not too many seedlings should be grown on a single plate since fungus mycelia from one or two of the seeds may spoil otherwise sterile seeds at some little distance on the surface of the medium. The infected seeds will be in the majority even with careful surface sterilization, and this fact should be realized in placing the seeds.
(2) Infection:

Infection was in general accomplished without wounding, usually without the addition of anything other than the suspension of spores or mycelium in water.

(3) Killing and embedding:

At first the killing solution used was an aqueous solution of chromic and acetic acids, made up as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromic acid</td>
<td>0.25 gm.</td>
</tr>
<tr>
<td>5% acetic acid</td>
<td>2 cc.</td>
</tr>
<tr>
<td>Water</td>
<td>100 cc.</td>
</tr>
</tbody>
</table>

This was found to be by no means so useful as a killing solution of absolute alcohol, chloroform and glacial acetic acid, as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute alcohol</td>
<td>6 parts</td>
</tr>
<tr>
<td>Chloroform</td>
<td>3 parts</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>1 part</td>
</tr>
</tbody>
</table>
The advantage of this killing solution is in the rapidity with which the sections can then be brought into paraffin, requiring only two days at the most, whereas the first process should have at least a week. This solution has been recommended by other workers for similar problems. (5,V)

The embedding process was as follows, using the second killing fluid:

(1) Specimen killed at the end of a day's work.
(2) Killing fluid (until next morning) 16 hours.
(3) 95% alcohol 1-6 hours.
(4) 100% alcohol (over night) 16 hours.
(5) Mixtures of 1 to 2 xylene and absolute alcohol, 2 to 1 xylene and alcohol, and pure xylene, from a few minutes to 15 minutes apiece. The pure xylene should be replaced once to ensure removal of the alcohol.
(6) Paraffin in a small lump added to the xylene and allowed to dissolve slowly until saturation is reached. This step of the process can be carried
out to advantage by closing the mouth of the tube containing the specimens and placing in a 37°C incubator. When convenient during the day the specimen can be heated in several changes of pure paraffin, poured into a box made of strips of paper, chilled quickly and sectioned. All specimens in the present investigation were cut 12μ in thickness.

If more than one piece of infected material is at hand the portions should be arranged in the mould before chilling in such position that one will be cut into longitudinal sections, the other into cross sections. By this means very favorable results may be secured in one way which would be much less noticeable in the other.

This process may be materially shortened, and even put into a single day if necessary, but two days is not an excessive period and is usually easier to arrange. The two nights are thus used to advantage to make sure of the important killing
and dehydrating processes.

(4) Staining:

The stains used were Delafield's Haematoxylin and a counterstain of eosin. The fungus takes the haematoxylin, the normal plant tissue takes both to some extent, giving a purplish red, injured portions take the eosin only and show a brilliant orange red coloration. Since injured portions are practically always in evidence in infected areas, either on account of the fungus or of the osmotic conditions prevailing at the point of inoculation, or on account of wounding with the needle, the eosin is by far the more important of the two stains in locating the disease. This is particularly true if a large number of sections must be examined as is usually the case in paraffin work; after the characteristic lesions are once observed and compared under all the powers available, additional examination to locate similar places in other portions of the material may be carried on with the
lowest power, showing a much larger field and avoiding fatiguing movements of the eye. For such work a forty millimeter lens is to be recommended in all cases in which it is reasonably sure that this magnification will furnish a detectable trace of abnormal conditions.

For rapid, temporary work it might be profitable to do away with the many operations involved in passing slides through different alcohol strengths to stain with water solutions, and to stain directly in the xylene used to dissolve away the paraffin. A little eosin added to this will stain the tissues enough in about one minute to greatly facilitate examination, and the specimen is ready to mount in gum damar. The use of this one bath instead of the round trip through all the alcohols and stains cuts down the time required to about one-fifteenth of its normal length. This fact was unfortunately not recognized in this investigation until all the work was complete and it was evident that the haematoxylin was not strictly essential.
EXPERIMENTS

During this investigation a few more than forty experiments were carried out, using organisms of three genera as follows:

- **Fusarium sp.** Strain I
- " " " II
- " " " III

**Alternaria sp.**

**Helminthosporium sp.**

**Source of Organisms:**

The first strain of Fusarium used was obtained in the form of a portion of infected head of wheat covered with the conidia of Gibberella saubinettii. The second strain was isolated from an infected seed in the laboratory in the form of large masses of conidia. This was of questionable character, and was probably a saprophytic species, but was used for comparison as the spores were so plentiful. The third strain was isolated from infected seed by Miss MacInnes,
who was working on this problem at the Institute at the time; the first strain was also obtained through her kindness.

The Alternaria and Helminthosporium were isolated from infected seeds which they killed on plates prepared for inoculation with the other fungi. They were used, not because they bore directly on the question in hand, but because the Alternaria seemed to be so persistent in attacking the wheat, and because Helminthosporium had been recently (1920) described as a cause of an important wheat disease. (8). It was hoped that work on these might point the way to technique capable of demonstrating entrance on the part of Fusarium.

Fusarium sp. Strain I:

During the series of experiments involving the strain of Fusarium from an infected head, the following points were brought out. Old tissues, whether from leaf or sheath, which become dry and wilted, are almost impossible to section because
of the air contained in the cells. It is impossible to drive this out by any moderate process of soaking or heating, and only by the use of an exhausting apparatus can liquids be forced in enough to allow final penetration of the paraffin.

Wheat infected without wounding, by placing these spores in a drop of water on the sheath covering the first leaf, was not harmed so far as could be discovered macroscopically, though this was repeated many times; later sections showed that no microscopic lesions had developed. Trials under different conditions of light and of temperature were made, but with no more success. The sections were perfectly normal, although browning of the tissues sometimes occurred. This was probably due to the presence of the material deposited on the sheath. Since infection had not been discovered in any of the sections thus far, stabbing with the needle carrying spores was tried with the hope that the spores would find it easier to germinate under these conditions. This
also failed, and suspicion began to be attached to the spores. While these were being tested the experiments with varied temperature and light conditions were repeated with the same result, and needle stabs were made into these still healthy plants. Results in all cases were negative.

The spores were tested as follows:

(1) Spores were mixed under a cover slip in a drop of water. A piece of wheat epidermis from the sheath was introduced, and the whole allowed to remain for a total of seven days, being examined frequently. No germination was noted.

(2) As soon as this method seemed to be at fault, a second test was made in which some air was admitted. Negative results were obtained as before. Germination was not observed.

(3) More air was used in a deep cell with a drop of water on the cover glass and a supply of water in the bottom of the cell. Negative result.
(4) Spores were placed in drops of water on the top of a Petri dish, and various reagents added with the spores, as follows: dilute acetic acid, dilute sugar broth. Ordinary water cultures were also tried without pieces of wheat epidermis. Germination was practically absent. It would be safe to say that not more than one per cent. of the spores showed signs of germination.

(5) A comparative test was run with the large numbers of spores of Strain II, isolated originally from an infected wheat seed, which were not typical of the organism producing wheat blight, but which were related forms. These sprouted under all the conditions tried in from twenty to twenty-four hours.

Conclusion from this series of experiments:

It was therefore concluded that the spores had been exposed to some unfavorable conditions and were unable to germinate. This was confirmed when an investigation was made; the spores had been kept in
diffuse light indoors throughout a considerable part of the winter.

The observations on their inability to germinate are therefore a confirmation of a recently published study of the effect of temperature and light on Fusarium (4). The substance of the work reported in this article as it affected this work was that diffuse light, especially indoors, but also outdoors to nearly the same extent, reduced germination to less than one percent. in the course of a winter.
Fusarium sp. Strain II:

Germination of these spores was found to take place in about 20 hrs. in drops of water supplied with air.

About one hundred young seedlings were inoculated twice on successive days with these spores, placing them in droplets of water on the sheath. No infection was observed. This experiment was repeated, with checks not inoculated, to rule out the infections sure to be found in most of the seed.

No difference between the inoculated seeds and the check seeds could be detected. From a repetition of this experiment a few seedlings were found to be relatively free from contaminating fungi and bacteria, as shown by examining the surrounding region on the agar plate and noting the absence of growths such as commonly are to be observed in the vicinity of surface sterilized seeds. These were isolated in sterile agar tubes and used in the next series of tests (those made with Strain III).
Since the sheath covering the first leaf of the seedling is provided with no stomata, it was thought best to give this strain, as well as the others, a chance to enter through the stomata of the first foliage leaf, though it has never been suspected of entering in this way. A number of shoots and leaves were inoculated with thousands of spores in water and allowed to remain for two days. A light, fluffy growth was especially noticeable on the leaf, and leaf spot seemed to have developed. Some specimens were cut and killed rapidly with the concentrated killing fluid; these were carried through paraffin, but showed no infection in sections.

To test the ability of these spores to send hyphae through the tissues after the death of the wheat, other sections were cut from leaf and sheath, and placed on agar plates for three days, at the end of which time growth of the fungus was noticeable. Sections of these specimens were somewhat puzzling for they showed complete infection
without lesions in the peripheral cells which could explain entrance.

More careful examination showed that the action on the cells was progressive, the cell walls which enclosed many mycelial threads became gradually thinner as the infection proceeded until in some sections they were barely visible and finally faded entirely away, leaving only the threads of mycelium to give a semblance of the cell structure by their positions along the lines of the former walls.

In a few places peripheral cells were penetrated, but in all cases the fungus seemed to be going out rather than in, as shown by the direction of branching and by the fact that the concentration of mycelium on the inside was much greater than that outside.

Within the sheath the mycelium seemed to have no difficulty in passing through the cell walls, leaving almost no trace of its attack, passing through without harm to the surrounding portions of the penetrated wall.
Injuries to these heavily infected cells were not shown by the eosin stain, if they were present. But in some of the sections the cell walls were suspiciously orange red and swollen at the points where normally there are intercellular spaces. Examination of these areas under high powers showed that the hyphae of the fungus, which stain deeply with haematoxylin, were passing up through the longitudinal intercellular spaces, enlarging these at times by crowding in, three or more threads in a single space. The walls in the immediate vicinity were thicker than usual and took the eosin as though injured.

A diagram of this condition is shown on the next page, followed by a normal cell of similar appearance, stained only with haematoxylin, but showing the normal intercellular spaces with the same magnification. Both of these diagrams are camera lucida drawings with an oil immersion lens. The total magnification is about fifteen hundred diameters as shown by the scales of microns attached.
Stained with Delafield's haematoxylin and eosin.

Saprophytic organism passing through intercellular spaces.

(30)
Normal sheath cell, showing intercellular spaces.

Stain: Delafield's haematoxylin.
No cases of entrance through the stomata were observed, though in many sections fungus and stomata were shown in good contact. The substomatal spaces were always clear and the surrounding cells in excellent condition.

Infection by this apparently saprophytic organism must have occurred by way of the cut ends of the cells at the point where the fragment was detached from the living plant. In three days plenty of opportunity was given for the fungus to grow the length of the piece, a matter of a centimeter or less, and up through the cells from this wounded and unresisting area.
Fusarium sp. Strain III:

Several seedlings which showed no organisms present when grown on agar, as determined by searching the surface of the medium in the vicinity with a low power of the microscope, were isolated in tubes of agar and inoculated with the spores of this strain. These spores were in an emulsion containing some mycelium as well. No infections resulted.

Three plates of seedlings which were much less contaminated than usual with fungi or bacteria were selected from a number of plates grown and used to inoculate further with this strain. Sectioning showed no infection. One further attempt proved negative also.

In order to test the ability of this fungus to enter stomata of the first leaf, and to penetrate dead tissues of the wheat sheath and leaf, an experiment similar to that carried on with the previously described strain was performed. Living leaves and sheaths were inoculated with emulsions
of the spores and killed after two days. Other similar portions were cut and allowed to remain three days on agar plates before killing with the concentrated killing fluid. Examination of a large number of sections showed no results from the inoculation.
Alternaria sp.:

A species of Alternaria was so frequently isolated from the wheat seed after surface sterilization, and was found to penetrate the epidermal cells so readily that it was decided to inoculate a number of times with this organism for the purpose of developing a technique sufficient to demonstrate lesions in the cells with ease and rapidity. This result was attained in the course of the work.

The spores were readily visible on the seedling under low powers of the microscope, and so were more readily watched than the Fusarium spores. The latter were found to be so transparent as to escape detection even when present in considerable numbers.

Within two days after inoculation of wheat seedlings with a number of these spores, the surface was found to be punctured in many spots, and whole mounts of thin pieces of the sheath were made. These were stained with safranin and mounted in glycerine.

The top views showed clearly that a large proportion
of the spores had sent out hyphae, and that these had succeeded in penetrating the long cells of the epidermis. Moreover it was noted that the penetrating tubes were not of the same diameter as the rest of the mycelium, but were of the nature of thin infection threads. These were usually of very short length, penetrating immediately under the point of departure from the main hypha.

It was not easy to see in these whole mounts, which could be viewed only from the surface inward, just how far these infection threads proceeded, nor how they penetrated. It was therefore necessary to section so as to see the process in profile.

The sections showed that the infection tube was surrounded by a cone-shaped area, strikingly changed by the proximity of the infection thread, and suggesting the result of enzyme activity. This area and the infection thread usually passed into the cell nearly to the opposite wall, sometimes passing through a nearby partition to another cell.
In the whole mounts many of these infection threads and their surrounding cone-shaped affected areas were observed after the hyphae and spores had become detached from them. All of the threads seemed to have penetrated for about the same distance irrespective of the time allowed for the process, as would be expected from the saprophytic character of the organism.

This suggests an interesting parallel with the observations of Shear and Wood in 1913 on weak parasites of the genus Glomerella (6). They guessed that some of the species must have germinated from the spore as soon as this reached the surface of the plant, sending out hyphae with appresoria, from which germ-tubes penetrated a short distance into the tissue. These were able to withstand more severe conditions than the spore, lightly attached to the surface and easily washed off by rain. They served the purpose of a resting stage, awaiting the death of the organism, and in a position to respond immediately in such a contingency.
The writers mentioned did not see this happen, but assumed it from the fact that pieces of tissue showing no spores developed the fungus on incubation, and from some observations on the surface of the specimens in which bodies were made out to fit this theory. The difficulty of observation in this way made the results uncertain.

Their microtome sections of specimens, from which the remnants developed the disease if incubated, showed no such conditions. They thought that this was quite natural because of the minuteness of the infection threads and because they would be hidden in the structure of the cells.

Evidently they did not use a differential stain, but only one which stained the whole section uniformly. Under these conditions it would not be surprising that they failed to see the tubes. But by using a stain such as eosin, with or without a background stained with haematoxylin the points of infection could hardly be missed. They would stand out as
bright orange red spots on a pale background.

The words of the authors mentioned were as follows:

(6)

"Many races of Glomerella under ordinary conditions appear to be rather weak parasites" ........."In the majority of cases the most probable explanation of the dormant infections which have been shown to be present in so many instances in leaves and fruit showing no external evidence of disease is that the conidia on ascospores germinate whenever they come in contact with the plant surface under favorable conditions of temperature and moisture, and produce appresoria which are able to endure more unfavorable conditions than the spores, and which in turn send a germ tube through the epidermis. This tube apparently penetrates at first but very short distance and does little harm to the host cells, remaining in a dormant or inactive condition until the host becomes weakened or injured or the organ infected dies a natural death. Bodies resembling appresoria have
been found on the surface of normal apples and leaves upon which the fungus developed in a moist chamber, and they are sometimes found in abundance on the surface of lemons and other citrous fruits. It is difficult positively to identify these bodies on a leaf surface and trace the germ tube in the tissue, and the writers have as yet been unable to devote the necessary time to this feature of the investigation to verify the suggested explanation of the facts observed.

Large series of microtome sections of presumably infected leaves, the unused portions of which developed the fungus when placed in a moist chamber, have been studied, but the presence of fungus hyphae has not been demonstrated with certainty. This would be quite natural if the supposition that the dormant infection is restricted to a short hypha or germ tube just penetrating the surface is correct."

The work done on Alternaria would seem to bear out to a remarkable extent the explanation offered for the dormant infections of apples and other fruits. It is
probable that if the sections of the latter were to be examined with differential stains it would be found that the appresoria do not exist, at least in typical form, but only the thin infection thread; for in the present investigation stained whole mounts gave very decidedly the effect of appresoria, but sections showed that the appearance was due to the modified area in the immediate vicinity of the infection tube.

This work bears also a relation of some interest to the investigations carried out by Stakman (2) on the relation between parasite and host in immune and non-immune species. It was found that the essential difference between an immune and a non-immune species was that the former was chemically unsuited to the parasite; the immediately infected cells died quickly, and in its turn the fungus suffered rapid changes sufficient to prevent its entrance into other cells. Presumably Alternaria is unable to proceed further into the cells of the wheat without setting up
destructive changes and reactions, local so far as the wheat is concerned, but disastrous to the final infection after the death of the tissue. It would seem as though the fungus had been adapted in the course of evolution to stop at just the right point and await developments. But this habit leaves it in extremely close contact with the cell contents, and it is probable that it is being slowly accustomed to their action by the selection of those individuals best able to withstand such an environment.

It would therefore seem to be on the road of evolution toward complete parasitism when a sufficiently resistant variety shall have been produced to enable it to do away with this resting period and to enter the living wheat immediately. Thus Alternaria would seem to be a parasite in the making. How soon such a process of selection could be completed is a doubtful question, perhaps in a short time if the fungus is very variable in chemical content; perhaps only in a very long stretch of time if it is relatively stable.
Drawing from whole mount of portion of sheath, showing entrance of Alternaria from germination of spore
Stained with safranin, mounted in glycerine.

(1-25-1)
Detail of similar mount, under oil immersion showing infection thread and surrounding affected area (1-29-1)
Longitudinal section
showing germinating spores, and infection tube
Eosin Haematoxylin Stain
(1-21-1)
Longitudinal section
showing infected spot in perspective

** * * * * * * * *
(1-21-2)

** * * * * * * * *
Longitudinal microtome section at surface:

Thickness twelve microns

Showing characteristic zones with eosin

Wall swollen near one point of infection.

(1-32-7)
Helminthosporium sp.:

Helminthosporium spores inoculated onto the sheaths of seedlings produced microscopic lesions in four days. A few sections were made of these. In the sections several lesions were shown. The fungus caused a remarkable swelling of the cell walls in the immediate vicinity, changing their chemical nature so as to cause a differential staining reaction. They showed a very bright red with eosin, against the background of purplish red of the normal walls stained in part with haematoxylin.

On the whole the series examined pointed to entrance between the cells and passage through the intercellular spaces. The fungus must secrete a substance capable of diffusing some distance from the point of attack, and of causing softening of the walls.

Diagrams showing the phenomena observed will be found on the following pages, the staining characteristics of the cell walls being represented by the color used in outlining them.
LESION CAUSED BY HELMINTHOSPORIUM.

Eosin Haematoxylin Stain

Camera lucida drawing, under oil immersion

showing swollen cell walls

(1-33-4)
DISCUSSION OF RESULTS

From the examination of several thousand paraffin sections of the sheath and leaves after inoculation with strains of Fusarium no evidence for the entrance at this stage could be found.

A negative statement such as this requires for practical proof a far greater number of trials than could well be carried on in the limited time allowed, and should have the benefit of virulent strains from infected areas. However, these experiments seem to point toward a resistance of the wheat in the early seedling stage to infection through the sheath covering the first leaf, and even through the early stages of the first leaf.

Former statements, claiming that the fungus is able to enter at these points may well be questioned somewhat carefully. Most of the examinations reported have been macroscopic in character, and might well be ascribed to some other cause. Some of the casual remarks of other workers show that errors may have
occurred.

The three types of possible errors are as follows. Plants surface sterilized, but not controlled by checks, have been reported as developing symptoms after inoculation on the sheath. But this will occur with most seeds, whether inoculated or not, in spite of attempted sterilization.

Plants controlled by checks have been inoculated by spraying spores over the sheath, incidentally inoculating all the other portions of the plant. Infections resulting from such treatment mean very little unless examination of the sheath by histological methods is made, and lesions correlated with the presence of spores in the vicinity.

Finally, lesions simulating leaf-spot may occur on account of physical injury to the tissues by the inoculation, without infection.

It would therefore seem wise, if further histological work were to be done on this general problem, to proceed in the following manner:

1) Strains of the organisms in as virulent a
condition as possible should be obtained.

(2) Inoculation should be made on coleoptile, coleorhiza, first foliage leaf, and permanent roots, and into the seed, with a larger number of checks than usual to determine whether the infection is really due to the inoculation.

(3) Fragments of leaf, root, and sheath should be as small as possible, not more than half a centimeter in length, should include the point of inoculation and some of the unaffected portion, and should be obtained in duplicate at least.

(4) Specimens should be killed in the concentrated killing fluid mentioned in this work, sectioned so as to give longitudinal and cross sections of each type of inoculation, and stained with eosin only if time limitations prohibit the use of haematoxylin.

(5) Examinations for lesions should be conducted with a 16mm. lens until some evidence is obtained that the stains are working well, and that the affected areas will show enough to be detected with
the 40mm. lens, when this should be used as a means of covering more specimens in a short period of time.
SUMMARY

(1) The strains of Fusarium used showed no ability to enter the living tissues of the sheath or of the first foliage leaf of young wheat seedlings.

(2) Alternaria sp. was observed to be a frequent weak parasite or saprophyte on the tissues of the sheath. It enters by a short infection thread which proceeds but a short distance into a single cell and then becomes dormant until the infected organ dies.

(3) Helminthosporium was observed to produce lesions involving swollen and modified cell walls. It seems to pass between the cells.

(4) Rapid methods for embedding and for staining paraffin sections of small pieces of infected plant tissues were used and described.
BIBLIOGRAPHY

(1) Fusarium Blight of Wheat and Other Cereals.
    Dimitr Atanasoff. Contribution from
    Wisconsin Agricultural Experiment Sta-
    tion, and Bureau of Plant Industry.
    Published in Journal of Agricultural

(2) A Study in Cereal Rusts. Stakman. Minn.
    study of resistant and susceptible
    strains.

(3) The Fusarium Problem. Wollenweber.
    Discussion of classification.

(4) The Effect of Temperature and Light on Fusarium
    sp. causing Wheat Scab. Jean MacInnes.
    Phytopathology 1920, X: 52.

(5) Studies in the Physiology of Parasitism. II and V.
    Blackman and Wellsford, and Dey.
Methods used are of value.

