THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Raymond Fogelman final oral examination for the degree of Master of Science . We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

May 27 1930

E C Stakman
Chairman

C. O. Rosendahl

H K Hayes

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Raymond Fogelman for the degree of Master of Science They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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THE HOST RANGE OF FUSARIUM SPP. CAUSING WHEAT SCAB

A thesis presented to the Faculty of the
Graduate School of the University of
Minnesota in partial fulfillment
of the requirements for the
Degree of Master of
Science

By

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INTRODUCTION

Wheat scab has been known for more than thirty years, although its seriousness, extent, and economic importance have only recently been recognized. The cultural characters and taxonomic position of the pathogenes involved have been carefully studied. But, aside from their identity with the scab of the other small grains and their connection with certain corn root and stalk rots, no extensive experiments have been made to determine the host range of the organisms involved. Since many Fusaria are known to have a wide host range, and, since the wheat scab pathogenes are members of this genus, the importance of determining their host range becomes obvious. It was with this purpose in view that the present investigation was undertaken.

HISTORICAL SUMMARY

Smith (32) first called attention in 1884 to a disease of wheat in England which he termed "wheat scab", and he named the causal agent Fusisporium culmorum W. Sm. A similar disease of barley and one of Lolium perenne he described as being caused by closely related forms. However, in 1869 Fuckel (13) attributed the disease of Lolium in Germany to Fusarium heterosporium Nees. This organism Kirchner (21) de-

scribed in 1890 as also causing a disease of wheat, oats, barley, rye, and maize.

Wheat scab in the United States was first mentioned by Chester (7) in 1890 as being serious in Delaware. Arthur (2) mentioned it as being important in Indiana in 1891, while Detmers (10) described Fusarium culmorum as causing wheat scab in Ohio. It was also mentioned by Pammel (24) as being troublesome in Iowa the same year. Buckhout (5) spoke of the disease as being abundant in Pennsylvania in 1892 and Bessey (3) reported that it was prevalent in Nebraska several years later.

Selby (28) suggested that Fusarium roseum Lk., of which Gibberilla Saubinetii (Mont.) Sacc. was probably the perfect stage, was responsible for the scab of wheat and oats.

Rostrup (26) was the first to show with any degree of certainty that seedling infection results from seed internally infected with Fusaria, and, according to von Tubouf (35), he also described Fusarium avenaceorum as a seedling parasite of oats in Denmark in 1893. Two years later Saccardo (27) described Fusarium culmorum (W.Sm.) Sacc. as the wheat scab pathogene.

Von Tubeuf (35) described F. heterosporium Ness. as being found on rye, maize and other Graminese and thought it was the same organism observed by Smith as parasitizing some of the cereals. He states further that Frank found a field of rye near Kiel, Germany, in 1892, completely destroyed and the ears quite overgrown by this fungus.

Frank (12) states that F. heterosporium Nees. occurs on the ears of certain cereals and grasses, but he considers this fungus saprophytic

upon the dead parts.

Von Tubeuf (36) described Fusarium lolii in 1908, occurring on Lolium perenne and other grasses, as being different from F. culmorum.

Delacroix and Maublanc (9) mention F. roseum Lk. as developing in the heads of cereals while Ferraris (11) gives Gibberella Saubinetii (Mont.) Sacc. as the perfect form of F. roseum Lk., the causal agent of "Golpe bianca," the scab disease of grains.

Undertaking a more comprehensive study of the whole problem, Selby and Manns in 1909 (29) reported successful cross inoculations of the scab organisms from wheat, oats, barley, and rye. They consider Fusarium avenaceārum, F. hordei, F. culmorum, and F. heterosporium as being the same organisms as F. roseum Lk. of which Gibberella Saubinetii (Mont.) Sacc. is the perfect stage. They demonstrated that the fungus persisted within the seed and attacked and destroyed the young seedlings. They also isolated pure cultures of F. roseum from alfalfa plants which were dying in the field.

In his handbook on plant diseases Selby (30) listed the following hosts of the wheat scab pathogene, F. roseum; alfalfa, barley, clover, corn, emmer, oats, rye, spelt and wheat.

Appel and Wollenweber (1) report rather extensive observations on an organism which they name Fusarium rubigenosum A. & W. They describe it as causing a rot of potato tubers. Later they determined this organism as F. culmorum, the wheat scab pathogene.

Lewis (22) made an extensive study of some disease-producing Fusaria in 1913. Among them was a culture which Manns had isolated from

scabby wheat and had identified as F. roseum. Wollenweber, however, called it F. reticulatum. Another culture which had been isolated from the glumes of wheat and which caused a rot of apples after artificial inoculation, was identified by Wollenweber as F. metachroum, while a third culture, which he identified as F. culmorum, was found causing a rot of squash in storage.

Johnson (19) reports a decided decrease in germination of wheat, oat, and barley seed inoculated with F. culmorum which had been isolated from wilted oats. No leaf spots resulted, however, from inoculating leaves of the cereals with the cultures.

Wollenweber (38) reports in 1914 that F. culmorum caused a rot of sweet potatoes. He carefully and thoroughly described the organism. Its cultural characters and taxonomic position are discussed and its distinction from Gibberella Saubinetii on the basis of the presence of chlamydospores is emphasized. According to Wollenweber "It is a wound parasite on cereals and causes scab and seedling blight (foot disease). It has been found on the following hosts: Zea, Avena, Triticum, Secale, Hordeum, Lupinus, Gossypium, Ipomea, Solanum, Cucumis, Cucurbita, and others."

In his memoir on the Fusaria of Potatoes published in 1915, Sherbakoff (28) also describes F. culmorum as causing a rot of the potato tuber and gives its cultural characteristics and spore measurements in full.

Harter, Weimer and Adams (14) obtained sweet potato rots as a result of inoculations with F. culmorum and G. Saubinetii.

Pammel, King and Seal (25) describe the wheat scab organism as causing the corn root rot as well as attacking the stalks and ears of the corn plant.

Hoffer and Holbert (15) state in 1915 that the same organism which causes wheat scab also causes a rot of the stalks and ears of corn. They also have observed that wheat planted in diseased corn fields had more scab than when following other crops.

The same year Hoffer, Johnson, and Atanasoff (16) report that cultures of G. Saubinetii isolated from corn roots and stalks were found to produce typical wheat scab. Similar results were obtained on both wheat and corn by using cultures from naturally infected wheat.

Holbert, Trost, and Hoffer (17) report that systems of rotation were found to affect considerably the amount of scab present. Wheat following two years of corn had a higher percentage of scab than when wheat followed wheat or when oats were introduced between corn and wheat.

Stakman (33) described the effect of the organism isolated from scabby seed on the roots of wheat seedlings grown in Sach's modified agar media. Inoculated plants were generally stunted and produced very short roots. The innermost part of the cortex and the vascular tissue of the roots were found to be most heavily infected, disorganization proceeding outward toward the epidermis.

Bisby (4) found that F. culmorum caused a slight rot of apples and a rather soft rot of potato tubers and cucumber fruit. No injury was noted when bean and pea plants were inoculated.

After a careful study of the cultures isolated from scabby wheat in Minnesota, MacInnes (23) concluded that the morphological characters of the spores agree more closely with the descriptions given for F. roseum than with those given for F. culmorum. The mycelium remained viable in the seed after several months of exposure to winter weather. The spores kept in the dark retained their viability for a longer period. All of the cereals and a number of wild grasses were infected with the wheat scab organism studied.

Johnson, Dickson and Johann (20) found G. Saubinetii to be the chief causal factor in the recent (1919) epidemic of Fusarium blight or scab of cereals. F. culmorum, F. avenacearum, and other Fusarium spp. were also involved, but to a lesser extent. At the same time Holbert and Hoffer (18) considered G. Saubinetii also as being the most common pathogene responsible for the root and stalk rot of corn.

OBJECT OF PRESENT INVESTIGATION

It is readily seen from the above summary that, although the wheat scab disease has received considerable attention, there are at least three main outstanding facts with respect to this problem that still require further investigation. The exact identity of the organism or group of organisms causing wheat scab is as yet a matter of doubt. The particular methods by which the fungi in question attack their hosts also requires further study. The exact host range, as determined by controlled inoculation experiments, has not, as yet, been fully investigated.

The purpose of the present study was to obtain additional information on the problems suggested above.

EXPERIMENTAL METHODS AND MATERIALS

All of the seed used in the following experiments was disinfected with 1-1000 solution of $HgCl_2$ and then washed with sterile distilled water. The soil and pots used were autoclaved for two hours under a ten pound steam pressure. For soil inoculation the fungus was first grown on steamed rice, which was then well mixed with the soil, sterile steamed rice being similarly used in the check pots. A sterile platinum needle was used to make the punctures in the stems and leaves. A bit of mycelium was then introduced into the puncture with the sterile needle.

The plants were then incubated for forty-eight hours immediately after inoculation. When the incubation period was less than forty-eight hours specific mention is made of the fact. The incubation chamber consisted of a large galvanized iron cylinder with a glass top. The cylinder fitted closely into a pan of water so that the air in the chamber was nearly saturated. The material to be incubated was then placed in a smaller pan inside of this larger one.

Upon removal from this incubation chamber the plants were placed in a large chamber with glass walls and top where a temperature ranging from 20°- 25°C. was maintained.

The following cereal varieties were used in this study, unless otherwise stated. Wheat, Marquis, Minn. No. 1239 (C.I.3641); oats, White Russian, Ia. 101½; barley, Manchuria, Minn. 105; rye, Rosen; corn, red flint, yellow dent.

The seed of the hosts not listed above was obtained from the Seed Laboratory of the Division of Plant Pathology and Botany of the University of Minnesota.

The sources of the cultures used in this study are given in Table I.

TABLE I.

LIST OF CULTURES USED

Culture number	Host from which isolated	Part of Host from which isolated	Remarks
200c	wheat	scabby kernel	Collected in New Jersey
600	"	" "	" " Missouri
1002	Hordeum pusillum	seedling	Blighted by culture 600
1003	pea	stem	Infected with " "
1004	cucumber	"	" " " "
1006	radish	seedling	" " " "
1012	flax	leaf	Soil inoculated with culture 200c
2004	corn	stem	Infected with culture 1004
2005	bean	pod	" " " "
2007	"	stem	" " " 74
2017	tomato	stem	" " " 1004
29	barley	scabby kernel	Obtained from Miss F. J. MacInnes
74	oats	seedling	Obtained from Miss F. J. MacInnes
2050*	wheat	scabby glume	Obtained from Miss F. J. MacInnes
F. martii	bean	root	Dry root rot - Isolated by Dr. G. R. Bisby.
F. lini	flax	root	Wilted plant - Grown on flax-sick soil, University Farm, St. Paul, Minnesota.

* Occurs as culture Gibb. in the plates.

INOCULATIONS ON POTATO, CARROT, AND APPLE

POTATO (Solanum tuberosum L.) The fact that F. culmorum is capable of causing a rot of the potato tuber has been demonstrated by a number of investigators (37) (31). To determine whether the organisms in this study could produce such rots four sound potato tubers were inoculated with culture 200c, (see Table 1) culture 600, Fusarium oxysporum, and F. trichothececides, respectively. A fifth tuber was retained as a check.

The tubers were washed, sterilized externally with 1-1000 $HgCl_2$ and rinsed in sterile distilled water previous to inoculation. A small plug was then cut out, under aseptic conditions, from each of the five tubers, a bit of the mycelium introduced, and the plug replaced. These tubers were then put under a bell jar and kept at a temperature ranging from 25° to 28° C.

The rotting of the tubers became evident five days after inoculation. The mycelium grew around the edges of the plugs and caused a slight discoloration of the tissues. Two days later the rot caused by F. trichothececides had extended about 4mm. into the parenchyma of the tubers. The other organisms did not cause such rapid rotting.

However, four weeks after inoculation the wheat scab organisms had produced a rot, 4 cm. in extent, into the very heart of the tubers. The rot produced by culture 600 was the more extensive of the two. Isolations made from this rotted tissue yielded pure cultures of the organism used in inoculating.

The other Fusaria had rotted the tubers equally well, the

most extensive of the four rots being produced by F. trichotheceoides. The rot produced by F. oxysporum was as extensive as that produced by culture 200c. The tuber retained as a check remained perfectly sound.

CARROT (Daucus carota L.) Using the same method of procedure, one carrot was inoculated with culture 600 and another retained as a check. Within a month the fungus caused a dry rot, 15 mm. in extent and dark brown in color. Tufts of pink and orange mycelium, bearing spores which resembled those of F. culmorum, were produced by the fungus on the rotted tissue. Pure cultures of the organism were isolated from these. No rot of any kind occurred in the carrot used as a check.

APPLE (Pyrus malus L.) Following the same method in detail an apple was successfully inoculated with culture 200c. Four weeks after inoculation a rot was produced similar to that resulting from Penicilium glaucum but lacking the concentric circles typical of such rots. The rate of rotting, too, was slower and the tissues were more sunken and wrinkled. The check showed no signs of decay.

INOCULATION EXPERIMENTS ON GROWING PLANTS

BEAN (Phaseolus vulgaris L.)

Burkholder (6) reports that a Fusarium, which he named F. martii phaseoli, is responsible for the dry root rot of the bean. Bisby (4) reported that he was unable to infect bean plants with a F. culmorum culture which he was using. It was therefore thought worth while to determine whether any of the cultures used in this study were capable of attacking this host.

Three pots, containing four seedlings each, were selected

fifteen days after planting. The soil of one of these was inoculated with F. martii, a second with culture 200c and the third was retained as a check. Soil inoculation was effected by removing some of the soil near each seedling, placing there a mass of the mycelium and spores from the culture, and then replacing the soil, care being taken not to injure the seedlings. The check pot was treated in the same manner, except that no mycelium was introduced.

No important differences between the plants in the three pots were discernible during the succeeding two months. Six weeks later, however, many of the leaves of the inoculated plants had become yellow and dropped off. The inoculated plants appeared less vigorous than did the plants in the check pot.

All of the plants were then dug up and the roots were examined and photographed. (See Plate I) The stems and roots of the plants inoculated with F. martii were discolored with large dark brown lesions, many of the roots having been killed and the whole system reduced. This disease resembled very closely the condition described by Burkholder (6) as dry root rot. The plants from the pot inoculated with culture 200c showed the same discolorations and reduction of the root systems, only to a slightly lesser extent. The stems, too, from both inoculated pots were commencing to shrivel and turn yellow. The stems of the plants in the check pot, it will be noted, were entirely normal and the root system, which was much better developed, showed no discolorations.

Two pots of bean seedlings, one containing two plants and

the other four, were selected about a month after planting. All of the stems and leaves of the plants were punctured with a sterile platinum needle. The four plants in the one pot were then inoculated with culture 600, the mycelium being spread over the punctured leaves and pressed into the stem punctures. The two plants in the other pot were retained as checks.

The mycelium made considerable growth during the incubation period and was fairly widespread over the punctured leaves. It also grew luxuriantly around the punctures in the stems, weakening the latter to such an extent that they fell over. Dark green water-soaked areas appeared around the points of inoculation. From these areas the mycelium spread rapidly up and down the stem. The leaves of the check plants, on the other hand, remained perfectly healthy. Twelve days after inoculation the infected plants were dead or dying, and the mycelium was spreading down the stem toward the roots. Pure cultures of the fungus were reisolated from the infected stems and leaf tissues. The two check plants produced normal pods. (See Plate II.)

This experiment was repeated, using culture 2050 (see Table I) for one plant and culture 1004 for the second, while two other plants were retained as checks. Only one puncture was made in each stem, whereas several punctures per stem had been made in the previous experiment. The results were exactly like those just described.

Several bean pods were punctured when about 1 cm. long and inoculated with the cultures listed below. Check punctures also were made. Excellent infection resulted in most cases. The mycelium of

culture 600 made a luxuriant growth and was rapidly spreading over the whole pod four days later. The tissues for about 3 mm. around the puncture became very dark green in color and developed a water-soaked appearance. Equally good infection resulted from the inoculations made with cultures 1002 and 1004. Culture 74 made a slightly poorer growth, while culture 29 and F. martii made no growth at all. The discoloration of the tissues, so evident in the infection of the previous cases, was also absent here. There was no visible effect upon any of the check pods. Reisolations of cultures 600, 1002, 1004, and 74 were made. All of the infected pods were killed and covered with the fungus a week after inoculation. The pods inoculated with culture 29 and with F. martii failed to develop further, although no mycelial growth was visible and efforts to reisolate these fungi were unsuccessful. The check pods remained healthy several weeks longer when all were discarded.

The results obtained thus far showed conclusively that stems, leaves, and pods easily could be infected through very small wounds. Experiments then were made to ascertain whether uninjured tissues could be infected. The leaves of several plants were moistened and the mycelium of culture 1002 carefully spread over the surface without injuring the tissues in any way. Some of the leaves were inoculated on the upper side, others on the lower side. A bean pod was similarly inoculated and a second one retained as a check.

Upon removal from the incubation chamber it was obvious that the mycelium had not only spread over larger areas of the leaves but

that it had actually grown through them. Pure cultures of the fungus were obtainable from the upper surface of those leaves that had been inoculated on the lower surface and vice versa. Brown blotches as well as little tufts of mycelium developed also on the inoculated pod. Several weeks later the inoculated pod became yellow and shrivelled, while the check pod remained healthy and produced normal seeds. (See Plate III) Pure cultures of the fungus were reisolated from both the inoculated and uninoculated sides of the pod, showing that the fungus had spread throughout the whole pod.

PEA (Pisum sativum L.)

Seedling peas were inoculated in the same way as described for bean seedlings. The soil of one pot was inoculated with culture 600, the other with culture 200c, while a third was retained as a check.

Ten days later the seedlings in both inoculated pots were beginning to show signs of wilting. Several of the plants were removed, washed, the surfaces sterilized in 1-1000 $HgCl_2$, rinsed in sterile distilled water, and placed on agar in a test tube. In a few days mycelium began to grow out (apparently from inside of the affected tissue) and a pure culture, indistinguishable from the one used in inoculating, was obtained. Culture 1003 was thus obtained from a plant inoculated with culture 600 and cultures 1035 and 1036 from those inoculated with culture 200c. The check plants remained green and vigorous. The experiment was duplicated and similar results were obtained.

Pea seedlings were transplanted January 17th into three small

pots and their stems punctured. One was then inoculated with culture 1003, the other with culture 1004, while the third was retained as a check.

The inoculated plants fell over at the point of inoculation on the day following their removal from the incubation chamber. These plants turned yellow and were beginning to die two weeks later. The check plant, on the other hand, remained vigorous, retained its green color and continued growing. (See Plate IV)

CLOVER (Trifolium repens.)

White clover was planted in two pots on December 31st. A good stand of clover seedlings was obtained in both pots a week later. One of these was then inoculated with culture 200c and the other retained as a check.

The fungus was cultured on steamed rice. When these grains were dry enough to be readily separable from one another, they were spread on the surface of the soil between the clover seedlings. Sterile steamed rice was similarly spread in the check pot. Both pots were then placed in the incubation chamber.

While in the chamber the fungus grew profusely and completely covered the surface of the inoculated pot. This surface was covered with a layer of sterile soil about 5 mm. thick a week later. The check pot was similarly treated.

Two weeks after inoculation it was quite evident that the fungus was killing many of the clover seedlings. Many of the stems and roots were entirely discolored, dark brown in color, and commencing to rot. Isolations made from the dead roots yielded the wheat

scab pathogene. Nearly half of the plants in the inoculated pot were killed, while the others were badly stunted.

The above experiment was repeated using culture 600. Even more striking and pronounced were the results obtained, although the difference was one of degree only. (See Plates V and VI.)

FLAX(Linum ustitatissimum.)

Using the method of procedure employed in inoculating clover, (see page 15) the soil of one pot of flax seedlings was inoculated with F. lini, another one with culture 200c, a third with culture 600, while a fourth was retained as a check. All were then placed in the incubation chamber. Two days later they were removed to a glass chamber where a high temperature (20°- 25°C) and humidity was maintained.

Some of the flax seedlings fell over a week after inoculation in a way similar to those plants affected with the damping-off organism. The roots were reduced in size, dark brown in color, and had the same general appearance as the clover seedlings described in the previous case. These plants were dead ten days after inoculation. The percentage of seedlings destroyed ranged from 5% in the pot inoculated with culture 200c to 15% in the one inoculated with F. lini.

The cotyledons of a number of the seedlings became infected several days after the soil was inoculated. This was especially noticeable on those plants whose germination had been delayed. This cotyledon infection occurred only in the three inoculated pots. Isolations from these infected areas yielded cultures indistinguishable from those used in inoculating.

TOMATO (Lycopersicum esculentum Mill.)

A series of experiments similar to those described for the bean plants also were made with tomato plants. Two pots were thus inoculated with culture 600, two with culture 200c, and two retained as checks. The effect of the fungus upon the seedlings began to show within a week after inoculation. Several of the seedlings in each of the four inoculated pots collapsed as though "damped off." The seedlings in the check pots remained turgid and healthy. Parts of the stems and most of the rootlets of the infected plants were deep brown in color, reduced in size, and were beginning to rot. Pure cultures of the scab organism were then isolated from these dead plants.

The stems of several tomato plants were punctured and inoculated with culture 600, the method being identical with that used for beans. The incubation period was limited to 36 hours. The inoculated plants fell over at the point of inoculation immediately after their removal from the incubation chamber. The area around the puncture was dark green, water-soaked, and nearly covered by the luxuriant growth of the fungus mycelium. (See Plate VII.) The water-soaked area had spread about 2 cm. in either direction from the point of infection, one week later, and the newly invaded tissues were beginning to shrivel and turn brown. The plants were dead several days later. The experiment was immediately repeated and similar results obtained.

To further substantiate these striking results a series of inoculations was started using various isolation and reisolation cultures as well as a number of Fusarium cultures which were kept in stock

in the Section of Plant Pathology, University of Minnesota.

Tomato plants were inoculated, as in the previous case, with the cultures listed below and incubated for 48 hours. The following observations were made two days later:

Culture 1002. Plant fallen over. Lesions and mycelial production as in the previous case where culture 600 was used.

Culture 1004. Same effect but to a slightly lesser extent.

Culture 74. Plant upright. Mycelium spreading around the point of inoculation. A lesion, about 2 cm. long, dark brown in the center, with a dark green border produced in the infected area.

Culture 29. Same as above but to a lesser extent.

Checks. Vigorous and healthy, the punctures beginning to heal over.

The plants inoculated with cultures 1002 and 1004 were dead three weeks later. Those inoculated with cultures 29 and 74 were still more or less upright although the lesions had spread considerably further up and down the stems.

A number of fibrovascular bundles dissected out in the area of the lesion were dark brown in color, contrasting with the green healthy fibers of the check plants. This discoloration extended for about 1 cm. into that part of the stem which was still green. A pure culture (2017) of the fungus used in the inoculation was reisolated from such a discolored bundle.

The experiment was repeated four times using cultures 1003, 1012, 1013, 1039, 2017 and 2050 as well as the cultures previously used.

The results obtained were identical in all cases. All of the inoculated plants fell over in a way similar to those shown in Plate VI. These results not only substantiated the previous findings but also showed clearly that these cultures acted as wound parasites.

The stems of several tomato plants were inoculated with F. lini, F. martii, culture 1012, and culture 600. Two plants were retained as checks. The plants inoculated with cultures 600 and 1012 had fallen over on the second day after inoculation and were dead a week later. However, those inoculated with F. lini, and F. martii, as well as the check plants, continued normal and healthy.

The leaves of a tomato plant were perforated, moistened and the mycelium of culture 600 spread over them. The fungus made a luxuriant growth, spread over the inoculated leaves, causing dark green water-soaked spots to appear here and there, especially around the perforations. These spots increased in extent, the centers becoming yellow and membranous within two weeks. It was obvious that the fungus was able to kill the leaf tissues and produce typical leaf spots.

Culture 1003 was carefully spread over some uninjured tomato leaves. Some were inoculated on the upper side, others on the lower side. The mycelium grew vigorously while still in the inoculation chamber and penetrated the leaves. Pure cultures of the organism were readily obtained from the lower side of those leaves which had been inoculated on the upper surface and vice versa.

Leaf spots, similar to those described in the previous experiment, began to appear several days later. These increased in extent

as long as the plants were kept in the warm chamber. Several healthy leaves which came in contact with the infected spots also became infected.

It was quite evident that these cultures not only could parasitize the wounded leaves but that under favorable conditions of moisture and temperature they also could attack and kill uninjured leaves.

RADISH (Raphanus sativus L.)

One pot containing five radish seedlings was inoculated with culture 200c, a second with culture 600, while a third was retained as a check, the method being similar to that employed in the soil inoculation of the bean.

Four of the seedlings in the pot inoculated with culture 600, and two in the one inoculated with culture 200c "damped off." Two weeks after inoculation their general appearance was similar to that described for the bean and tomato seedlings. Pure cultures of the organism were reisolated from these. The remaining seedlings in the inoculated pots, as well as those in the check pot, maintained their normal growth.

SUNFLOWER (Helianthus annus L.)

Fourteen sunflower seedlings, which had been planted February 16th, were transplanted two weeks later, one to a pot, the stems were punctured and inoculated with the following cultures: 29, 74, 200c, 600, 1002, 1003, 1004, 1006, 2050, F. martii and F. lini, while three were retained as checks. Only those inoculated with F. martii and F. lini and the three check plants remained erect on the

fifth day after inoculation. The others had broken at the point of infection in the same way as the tomatoes shown in Plate VII. The symptoms, too, were similar to those described for the tomato plants. Plate VIII shows most of these seedlings two weeks after inoculation. Only the three checks and those inoculated with F. martii and F. lini were still alive.

CUCUMBER (Cucumis sativus. L.)

Cultures 200c and 600 caused a "damping-off" of cucumber seedlings similar to that of the bean and tomato seedlings grown in soil infected with these cultures.

The stems of several cucumber plants were then punctured. Half of these were inoculated with culture 600, the remainder were retained as checks. Shortly after removal from the incubating chamber the inoculated seedlings broke over at the point of inoculation. These were dead two weeks later. (See Plate IX) Identical results were obtained when the experiment was repeated, even though the incubation period was limited to 36 hours.(See Plate X) The experiment was repeated a third time using the following cultures: 74, 200c, 600, 1002, 1003, 1004, 1006, 2004, 2005, 2017, F. lini, and F. martii. Twenty-five plants were inoculated and five were kept as checks. All of the plants inoculated with cultures isolated from scabby grain fell over and were killed within two weeks after inoculation, while those inoculated with F. lini and F. martii were as vigorous and healthy as the check plants.

This difference between the parasitism of F. martii and F.

lini as compared with the Fusaria isolated from the scabby grain was most obvious and striking throughout the whole series of experiments.

SQUASH (Cucurbita maxima)

Lewis (22) found that F. culmorum caused a rot of squash in storage. Inoculations were therefore made to determine whether the cultures used in this study could parasitize squash plants. The stem of a squash plant was inoculated with culture 2050. Punctures were made at the leaf bases of another plant. One of these was then inoculated with culture 1002, the other with culture 1006, and the third with F. lini. Both the stem and leaves of a third plant were similarly punctured and used as a check.

The plant inoculated with culture 2050 fell over at the point of inoculation on the third day following removal from the incubation chamber, and was killed by the fungus a week later. The leaves inoculated with cultures 1002 and 1006 also lost turgidity, dropped at the point of inoculation and wilted. The fungi used in inoculating were reisolated from these tissues. F. lini, on the other hand, was unable to parasitize this host. The inoculated leaf remained as turgid and healthy as those of the check plant. (See Plate XI)

CORN (Zea mays L.)

Several investigators (15), (16), (17), (18), have called attention to the fact that wheat scab was in some way intimately connected with corn root rot. Experiments therefore were made to determine whether the wheat scab cultures used in this study could produce this root rot.

Following the method used in the soil inoculation of the bean one pot containing seven corn seedlings was inoculated with culture 200c and a similar pot of seedlings retained as a check. During the next month the seedlings in the check pot grew much more vigorously than those in the inoculated pot. The plants were then transplanted to larger pots, the inoculated plants being reinoculated with culture 200c. All were then grown to maturity.

The inoculated plants became stunted as compared with the checks. (See Plate XII) There was an average difference of 15 cm. in height between the two series of plants. The inoculated plants also produced tassels two weeks later than the checks. Many of the roots of inoculated plants were dark brown in color, shrivelled, and commencing to rot, while those of the check plants were normal in every respect.

To study this root rot more carefully several pots of corn seedlings were inoculated with culture 600 and an equal number retained as checks. All of the roots were examined a month later.

The primary roots of the plants grown in the inoculated soil were dark brown in color, shrivelled, and dead. Many secondary roots had been produced and a number of these were similarly affected. Isolations made from these lesions yielded pure cultures of the scab organism. The roots of the check plants, however, were perfectly healthy, larger in number, and better developed. No discolorations of any kind could be found on them. (See Plate XIII)

SEED INOCULATIONS

Johnson (19) reports reduced germination of wheat, oat, and barley seed which had been inoculated with F. culmorum before planting. To observe more closely just what was taking place, some moist cotton was placed in a number of test tubes which were then plugged and autoclaved. Six plump wheat kernels were then sterilized in 1-1000 $HgCl_2$, rinsed in sterile distilled water, and planted on the moist cotton in two of the above test tubes, i.e. three kernels to each tube. One of the tubes was then inoculated with culture 600 and the other left uninoculated.

Two weeks after inoculation the three kernels in the check tube had all germinated and had produced seedlings about 6 cm. in height. Two of the kernels in the inoculated tube also had germinated and had made nearly as good a growth as the check seedlings in spite of the fact that the fungus mycelium was spreading over several of the roots and part way up the stem. The third kernel had not germinated at all but was completely covered by the fungus mycelium.

The check seedlings made a considerable growth during the succeeding week, while the growth of the two seedlings in the inoculated tube was very much reduced. The latter also began to turn a sickly yellow, the mycelium having spread over more than half of each plant. Their roots, too, were reduced in size and discolored by light brown and pink spots, obviously being parasitized by the fungus.

Two weeks later both seedlings in the inoculated tube were dead and covered by a luxuriant growth of mycelium. The seedlings in

the check tube, however, had continued growing and retained their green color, in striking contrast to the yellow and dead seedlings in the infected tube.

Identical results were obtained when barley, oat, or rye seed was used, excepting that all the inoculated oats were parasitized before they had an opportunity to germinate.

THE PRODUCTION OF SCAB

It has been demonstrated in the preceding experiments that the scab organisms used in this study not only could produce the root rot of corn but that they also reduced the germination of cereal grains and were capable of producing the seedling blight of cereals. It was now necessary to show that these cultures could also produce typical scab of wheat and other cereals.

Wheat heads which had been cut off at the time of blooming were inoculated, in a preliminary test, with the following cultures: 1003, 2004, 2017, 2050, and 29. Two heads were retained as checks. Plate XIV shows these heads three weeks after inoculation. The mycelium had grown luxuriantly, covering each head completely, including the sheaths in several cases. These infected heads soon turned yellow, although the checks were still green.

Marquis wheat was grown to maturity and the heads inoculated with cultures 1003, 1004, and 600. A fourth head was retained as a check. Inoculation was effected by spreading some mycelium over a number of the florets at the tip of the spike. The inoculated plants were kept in the incubation chamber for 48 hours and then removed to the large chamber

where a temperature ranging from 20° to 25° C. was maintained.

Typical scab was produced within two weeks after inoculation. (See Plate XV) The experiment was repeated using cultures 1002, 1003, 1004, 1006, and 2050, and identical results obtained.

Khapli is a variety of emmer that has been shown to be exceedingly resistant to a number of cereal pathogenes. Two plants of this variety were therefore inoculated with cultures 2005 and 2017 respectively. Typical scab resulted, the mycelium spreading gradually down the spike destroying the heads completely. (See Plate XVI)

The spikelets of one plant of oats were inoculated with culture 1004 and those of another plant kept as checks. The mycelium spread luxuriantly over the infected florets, gluing some of them together and causing them to turn yellow two weeks after inoculation. The spikelets of the check plant were normal in appearance and green in color. (See Plate XVII) The experiment was repeated using cultures 74, 1002, 1003, 1006, and 2050, and identical results were obtained.

The heads of barley plants were inoculated with cultures 1002, 1004, and 2050. A fourth was retained as a check. Within two weeks the inoculated heads were yellow, and completely parasitized by the fungus. The check plant was normal in every respect. The experiment was repeated and identical results were obtained. (See Plate XVIII)

This shows conclusively that the Fusaria which infected a variety of unrelated host plants were cereal scab organisms.

TABLE II.

Results of Inoculation with *Fusarium martii*, *F. lini* and *Fusarium spp.* causing Scab.

Host	Part Inoculated	Scab Organisms 200c	600	*F. martii*	*F. lini*
Bean	root	*		*	
Bean	stem		*		
Bean	pod		*	-	
Pea	seedling	*	*		
Pea	stem		*		
Clover	seedling	*	*		
Flax	seedling	*	*		*
Tomato	seedling	*	*		
Tomato	stem	*	*	-	-
Radish	seedling	*	*		
Sunflower	stem	*	*	-	-
Cucumber	seedling	*	*		
Cucumber	stem	*	*	-	-
Squash	stem		*		-
Corn	root	*	*		
Cereals	seedling	*	*		
Cereals	scab		*		

Legend

Successful infection *

No " -

DISCUSSION AND CONCLUSIONS

The Fusaria principally used in this study were isolated from scabby wheat. These consist of two groups; culture 600, and culture 200c, and their respective derivatives. The two series resemble each other very closely and conform to the general description given by Wollenweber (38) and Sherbakoff (31) for F. culmorum. They differ from each other, however, in two respects:-

1. Culture 600 is by far the more vigorous and active parasite of the two.

2. Under identical conditions, culture 600 produces considerably more red and orange pigment in the aerial mycelium than does culture 200c.

Although these differences are consistent, they are not differences in kind but in degree only.

From the historical summary it is clearly seen that there remains considerable doubt as to the exact identity of the species of Fusarium which cause scab of cereals. As a matter of fact, it appears quite probable that several species can cause the disease. The organisms used in this study conform most nearly to the description of F. culmorum as given by Wollenweber (38) and Sherbakoff (31).

The strains of Fusarium designated as scab-inducing Fusaria in this paper clearly can cause scab, since they originally were isolated from scabby wheat and subsequently produced scab when cereals were inoculated with them.

This capacity to attack all cereals, however, does not represent, by any means, the host range of these pathogenes. The writer has demonstrated conclusively that when these organisms are present in sufficient numbers in the soil they attack and destroy tomato, pea, clover, radish, flax, and cucumber seedlings. They also cause a dry root rot of the bean, similar to that described by Burkholder (6) and attributed by him to F. martii phaseoli.

It has been pointed out by Holbert and Hoffer (15) and others (16) that the scab pathogene also is responsible for the root rot of corn. The organisms used in this study also produced root rot of corn.

But even this does not exhaust the number of diverse hosts that these scab pathogenes can attack. It has been shown repeatedly by the writer that these organisms are exceedingly virulent wound parasites capable of attacking bean, pea, tomato, cucumber, squash, and sunflower plants. Furthermore, they not only have parasitized injured bean and tomato leaves and bean pods, but they also have infected healthy tissues of these plants.

It is obvious that we must revise our ideas about the parasitic capabilities of these Fusaria. The fact that they may enter such slight wounds as are produced by insects so universally, that they parasitize such diverse crops as outlined above, the widespread occurrence of these organisms, and their virulence combine to make them unusually insidious and dangerous parasites. Few plant pathogenes are capable of attacking such a variety of taxonomically unrelated host plants; few destroy the infected plant parts so quickly and completely. The fact that they

are facultative parasites makes them still more dangerous. During conditions unfavorable to their development on growing plants they are able to multiply on dead plant parts, or in the soil. It is therefore extremely important to determine the conditions under which they can become virulent parasites.

It is quite essential, first of all, to determine the specific identity of the various Fusaria which occur on crop plants. Furthermore, it is imperative, in order really to understand their parasitism, to ascertain their degree of constancy. It is quite possible that they may change rather quickly in response to environmental conditions. It has been shown by many mycologists and plant pathologists that the virulence of certain fungi is influenced profoundly by their host associations. It is not beyond the pale of possibility, therefore, that a given species of Fusarium, which originally is capable of attacking cereals, may become a weak wound parasite on beans, and, as a result of continued association with this plant, eventually develop the capability of normally parasitizing it.

It has been shown clearly that the activities of these Fusaria and their methods of attack are in a measure differential. It has also been shown that although the scab organism is capable of parasitizing many plants, it did not attack flax seedlings as severely as did F. lini, nor did it produce as serious a root rot of the bean as did F. martii. On the other hand, neither F. martii nor F. lini infected the cucumber, sunflower, or tomato plants, nor did their presence mechanically occlude

the vascular system as did the mycelium of the scab organisms. (See Table 2.)

It is axiomatic that moisture, light, and temperature affect very markedly the cultural characters and the parasitism of many fungi. It is quite probable that these factors also affect Fusaria. In fact this has been demonstrated for certain species. It is very desirable, therefore, that exhaustive investigations be made to ascertain the optimum environmental conditions for the development of these Fusaria.

The ultimate bearing that the life habits and constancy of strains of Fusarium which develop on crop plants may have on systems of crop rotation and sanitation may be extremely important. Soil sickness is a common phenomenon. Species of Fusarium are known to be responsible for several different kinds of soil sickness. It is obvious, therefore, that the value of extensive investigations of the biology of these pathogenes in relation to their parasitic habits is incalculable.

The control measures for scab are quite imperfect. Up to the present time these efforts have mainly been directed toward the elimination of diseased seed, and the introduction of proper crop rotation. However, since some of these organisms have been shown to have such a wide host range, our ideas of the efficacy of these rotations may have to be revised. The epidemic nature of these diseases also means that more attention will have to be given to these sources of inoculum.

It appears quite probable to the writer that there exists a distinct difference in the virulence of these organisms from different localities. If this is true, control measures will have to become quite as much a local problem as the development of disease resistant varieties of some crop plants.

At any rate, it is obvious that our present control measures are quite inadequate in the light of the facts brought out in this study. The scab epidemic of 1919 substantiates these conclusions. Further studies of these organisms and the factors that influence their multiplication and virulence are absolutely necessary before adequate methods of control can be perfected.

SUMMARY

1. Two cultures of Fusarium, both of which conform to the description given by Wollenweber and Sherbakoff for Fusarium culmorum, (W. Sm.) Sacc. were isolated from scabby wheat.

2. That these are typical scab producing organisms is conclusively proven by the fact that they were isolated from scabby wheat and produced scab upon reinoculation.

3. These organisms reduced the germination of cereal seed and produced seedling blight.

4. They induce a root rot of corn, as well as a dry root rot of bean similar to that produced by Fusarium martii.

5. The scab organisms rotted apples, carrots, and potato tubers.

6. When inoculated into the soil they attacked and killed bean, pea, clover, tomato, radish, and cucumber seedlings.

7. These organisms attacked and destroyed bean, pea, tomato, cucumber, squash, and sunflower plants when introduced into wounds.

8. Neither F. martii nor F. lini were parasitic under similar conditions.

9. Several different species of Fusarium can cause the scab of cereals.

10. The cultures isolated from wheat collected in different localities differed consistently in their parasitic capabilities.

11. Further suggestive evidence that the virulence of these organisms differs in different localities has been furnished during the last epidemic of wheat scab.

12. These studies have demonstrated conclusively that the host range of these organisms is much wider than had previously been known.

13. This wide host range has an important and direct bearing on the present methods of control wherein crop rotation figures so prominently.

14. The facts brought out in this study show the imperative necessity for determining the identity of the scab organisms, their morphologic and pathologic constancy, the conditions under which they develop, as well as their exact host range.

15. Adequate control measures for wheat scab must await further detailed studies of the life habits and characteristics of the various causal organisms.

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EXPLANATION OF PLATES

Plate I. Dry Root Rot of Bean

Check - Control plants; soil uninoculated.
200c - Soil inoculated with Culture 200c.
F. m. - Soil inoculated with *F. martii*.

Plate II. Stem Rot of Bean

Plants in pot on left, inoculated with culture 600.
Note stem punctures in check plants in pot on the right.
Arrows indicate points of inoculation.

Plate III. Dry Rot of Bean Pods

Compare shrivelled pod (marked with arrow) with the check pod on the plant to the right.

Plate IV. Stem Rot of Pea

Check plant, on the right, green.
Infected plants yellow and dead.

Plate V. Seedling Blight of Clover

Soil in pot to the right made "sick" with scab organism.

Plate VI. Seedling Blight of Clover

Same as Plate V.
Top view.

Plate VII. Stem Rot of Tomato

Four days after inoculation.

Plate VIII. Stem Rot of Sunflower

Infected plants all dead.
The first and seventh from the left are check plants, the third was inoculated with *F. lini*.

Plate IX. Stem Rot of Cucumber

Two weeks after inoculation.
Plants in the pot to the left, inoculated with culture 600. All dead.

Plate X. Stem Rot of Cucumber

Four days after inoculation.

Plate XI. Stem Rot of Squash

Pot 1. a. Leaf inoculated with F. lini.
b. Leaf inoculated with culture 1002.
c. Leaf inoculated with culture 1006.
Pot 2. Stem inoculated with culture 2050.
Pot 3. Check.

Plate XII. Root Rot of Corn

Pot 1 and 4. Soil inoculated with culture 200c.
Pot 2 and 3. Check plants.

Plate XIII. Root Rot of Corn

Compare the development, rotting, and discoloration of the inoculated roots as compared with those of the check plants.

Plate XIV. Scab on Wheat

Heads cut off from plants previous to inoculation.

Plate XV. Scab on Heads of Marquis Wheat

Check plant green.
Tips of infected plants yellow and glued together.

Plate XVI. Scab on Heads on Khapli Wheat

Heads yellow.
Note development of mycelium.

Plate XVII. Scab on Oats

Panicle of check plant green, those of inoculated plants, yellow.

Plate XVIII. Scab on Barley

Head of check plant, green; the others yellow. Note mycelium develop on infected plants.

PLATE I.

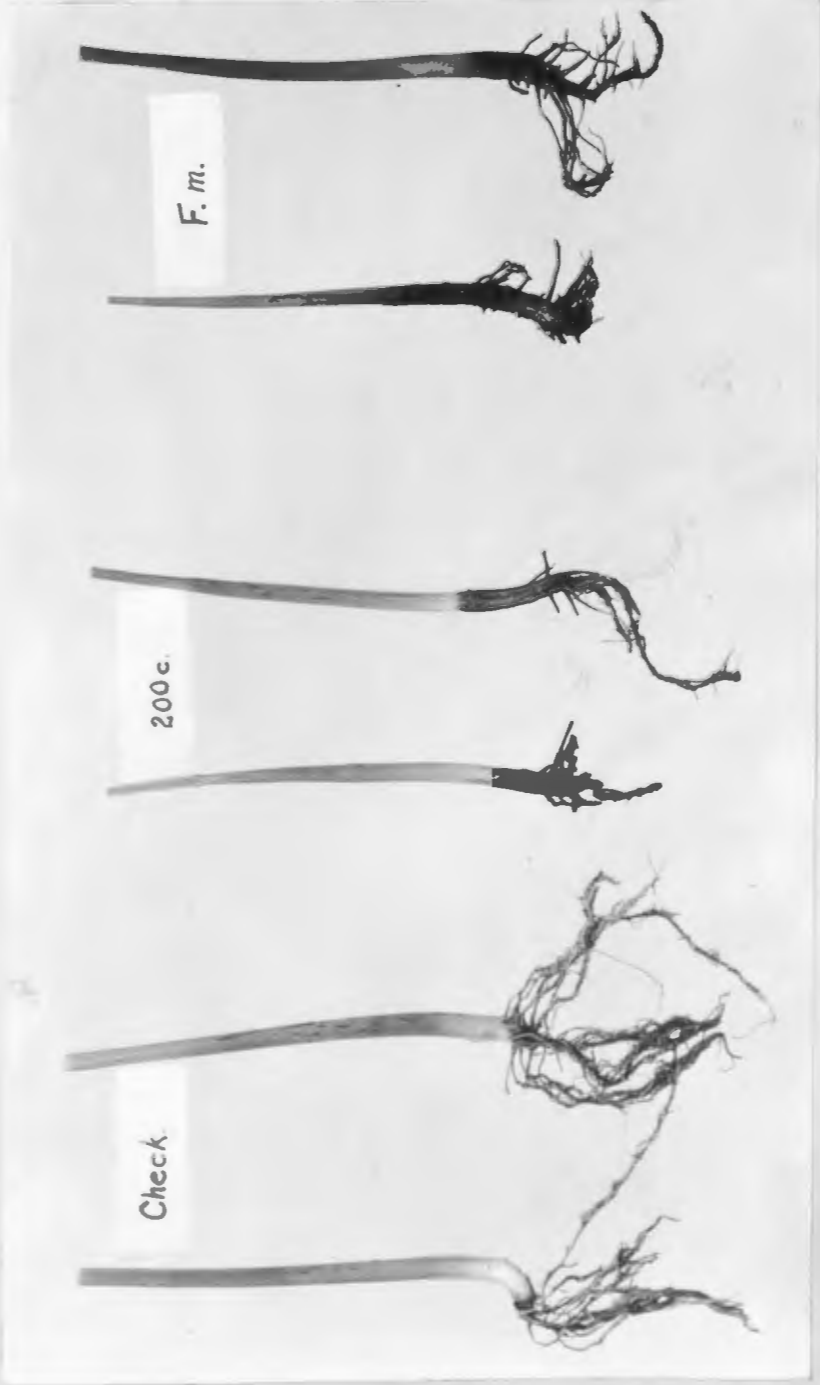

PLATE II.

PLATE III.

PLATE IV.

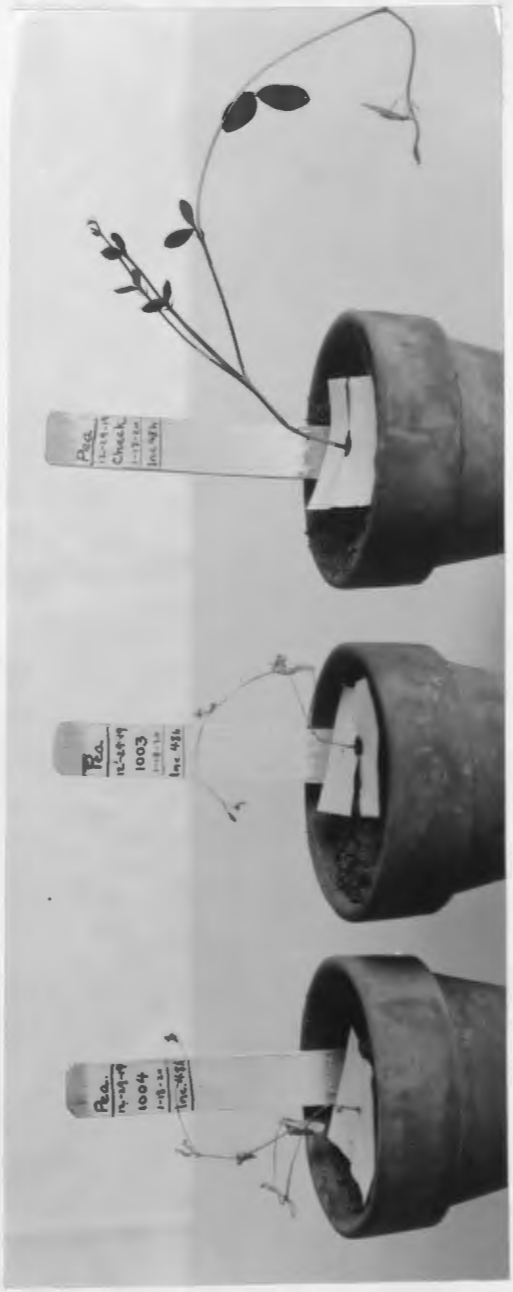

PLATE V.

PLATE VI.

PLATE VII.

PLATE IX.

PLATE VIII.

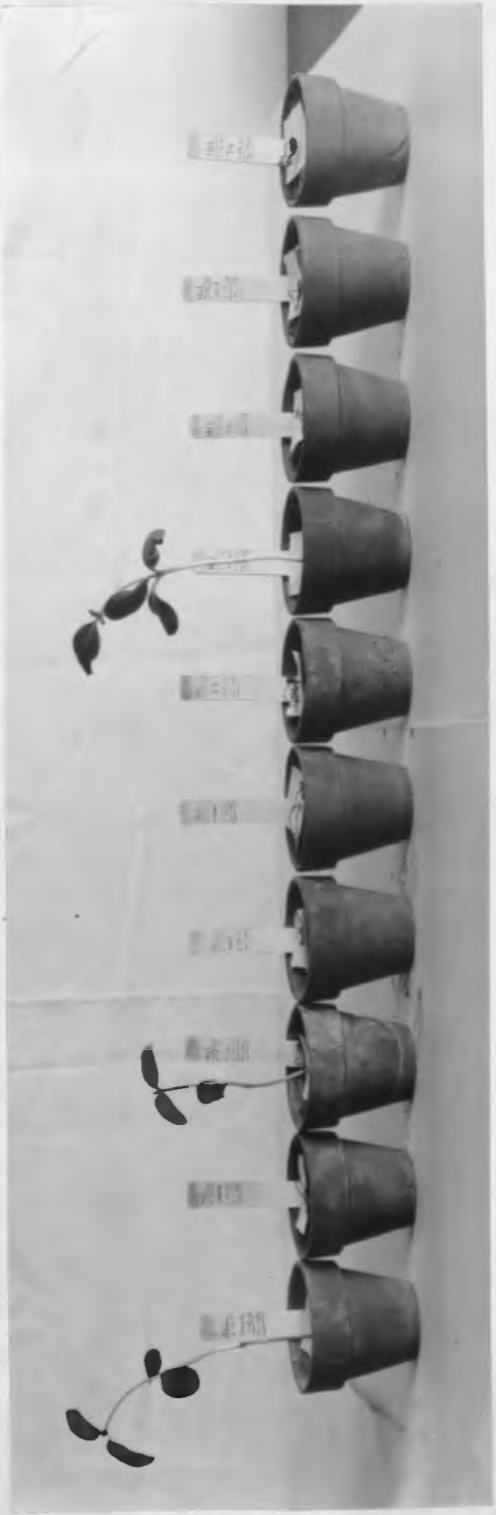

PLATE X.

PLATE XI.

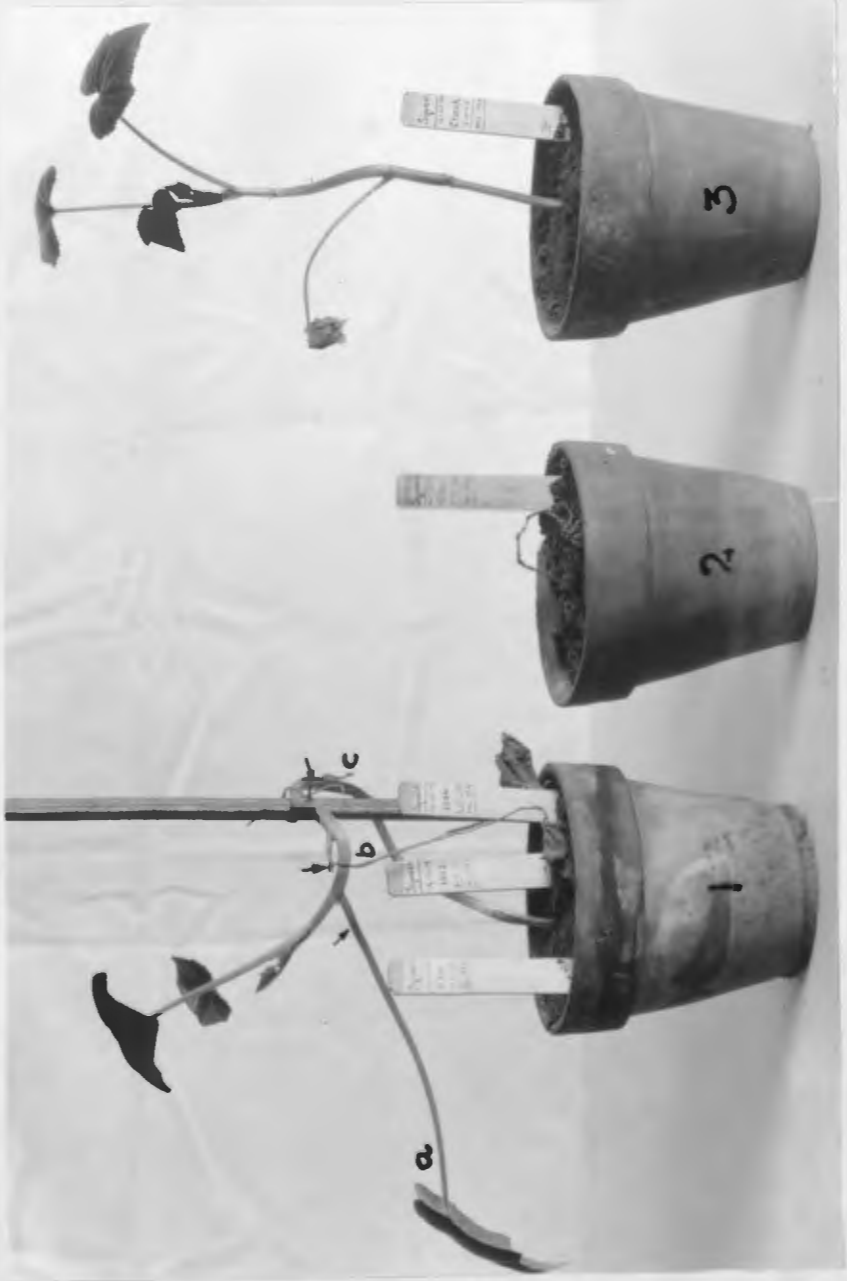

PLATE XII.

PLATE XIII.

PLATE XIV.

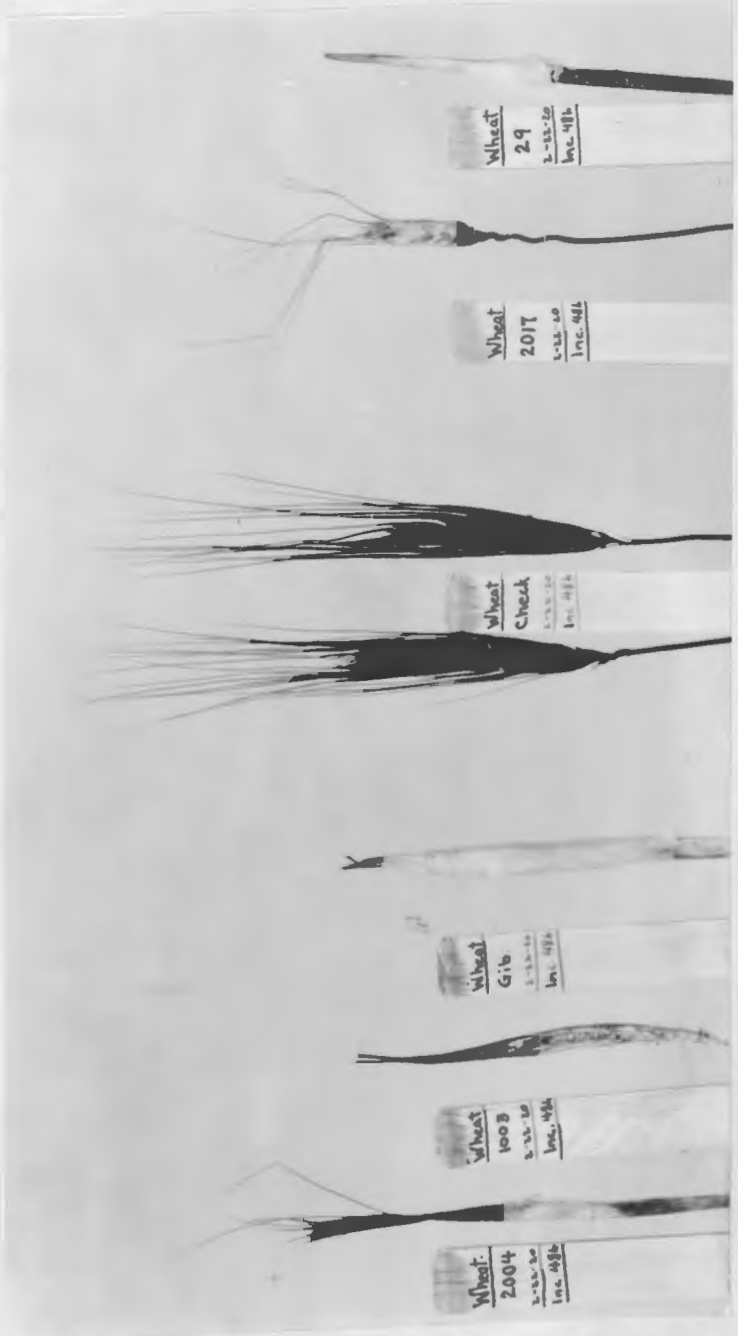
Wheat 29
Wheat 2017
Wheat Check
Wheat Gib
Wheat 1003
Wheat 2004

PLATE XV.

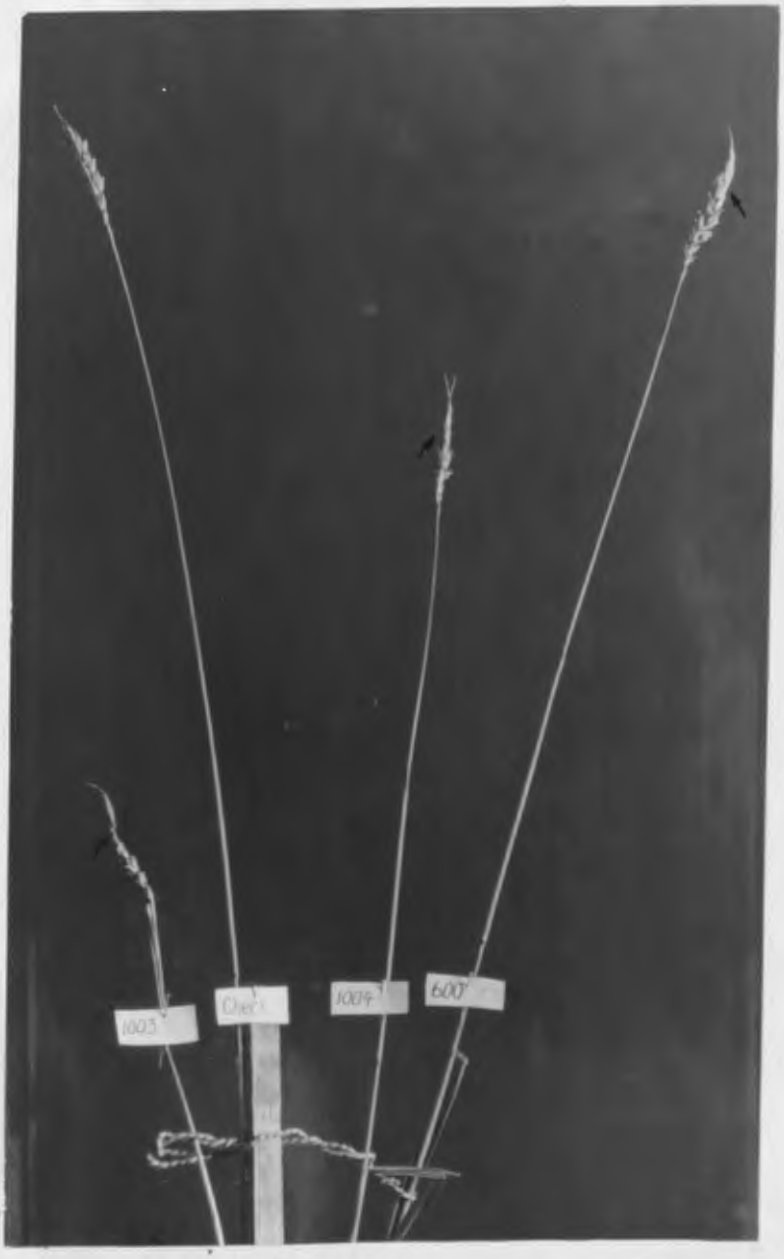

PLATE XVI.

PLATE XVII.

PLATE XVIII.