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Fusarium Blight of the Soy Bean and the Relation of Various Factors to Infection

Richard O. Cromwell

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FUSARIUM BLIGHT OF THE SOY BEAN AND THE RELATION OF VARIOUS FACTORS TO INFECTION

By Richard O. Cromwell

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FUSARIUM BLIGHT OF THE SOY BEAN AND THE RELATION OF VARIOUS FACTORS TO INFECTION

BY RICHARD O. CROMWELL

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During the summer of 1915 and each succeeding summer, packages of diseased plants of the soy bean *Soja max* (L.) Piper (20)[2, 3] were received at the North Carolina Experiment Station from several correspondents. A large number of plants in the fields from which these specimens were taken had become stunted or chlorotic, or were dead. The plants received were still green and in good condition for examination. The evidence obtained from a preliminary inspection indicated that the diseased condition was due to the presence of a fungus belonging to the genus *Fusarium.* Furthermore nearly all of the isolations from this material gave apparently pure cultures of a species of *Fusarium.*

Because of the importance of legumes in the cropping systems of the Piedmont and Coastal Plains sections, and because of the seriousness and extent of *Fusarium* diseases of members of this and thirteen other plant families,[4] an investigation was outlined (1) to determine the parasitism of this species of *Fusarium* on soy bean, (2) to establish its relationship to *Fusaria* of the section *Elegans* in so far as a comparison of the cultural characters permitted, and (3) by means of cross inoculations and field studies to determine the relationship of this disease of soy beans to the wilt disease of cowpeas (*Vigna sinensis* Hassk.) caused by *Fusarium tracheiphilum* Smith.

The results of these investigations up to the close of the summer of 1916 have been reported by the writer (6). The studies were continued at the North Carolina Experiment Station until

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[1]Formerly assistant plant pathologist, North Carolina Experiment Station. The writer is indebted to the North Carolina Experiment Station for leave of absence in order that full time could be given to these studies. Submitted for publication June, 1918.

[2]Reference is made by number to "Literature Cited," pp.

[3]Piper (20) gives the following as the full synonomy of the soy bean:

Phaseolus max L.
Dolichos soja L.
Soja hispida Moench
Soja japonica Savi
Glycine soja Siebald and Zuccarini
Soja angustifolia Miguel
Glycine ussuriensis Regel and Maack
Soja max (L.) Piper

[4]Wollenweber (31, p. 35)

the fall of 1917 and then at the University of Nebraska. A revised report of them is given in this paper. Additional studies of the soy bean blight, the results of which are also reported here, were planned to determine the effect of various factors on the amount and severity of the disease.

The writer wishes to express herein his sincere thanks to Doctors F. A. Wolf and E. M. Wilcox for their assistance and criticism during the time these studies were being conducted in connection with their departments.

ECONOMIC IMPORTANCE OF THE SOY BEAN

The soy bean is a native of tropical Africa, Asia, and Australia (26, p. 360-361; 20, p. 76) and was introduced into Europe by Kampfer about 1690 (21, p. 9). At the present time it is the most important legume grown in Japan, China, and Manchuria. The soy bean is the chief source of protein in human food in Japan, where very little meat, except fish, is eaten as compared with the amount of meat consumed in this country. Its culture in England was begun in 1790. The plant was introduced into the United States from Japan in 1860. Since that time its cultivation as a soil-improving and a forage crop has been confined for the most part to the Southern States. North Carolina is probably foremost among these States in the production of soy beans. The yield in 1909 was only 13,313 bushels (29, p. 632), and in 1915 was estimated[1] as approximately 1,000,000 bushels. Within the last three or four years, and especially since the war began, this crop has become increasingly important because of the large variety of products manufactured from the oil and meal and because of its introduction in the United States as a human food.

The following is a list of the most important products obtained from soy beans or in which soy beans enter: Soy bean milk, vegetable cheese, meal or flour, macaroni preparation, soups, pork and beans, meat substitutes, toilet powder, fertilizer, and cattle feed from the meal, and high explosives, soaps, linoleum, rubber substitutes, margarine, Japanese sauce, paints, varnishes, water-proof cloth, salad oil, lubricants, and lard substitutes from the oil.

The grain is more valuable as a supplementary feed than cottonseed meal for the production of pork, mutton, beef, wool, milk, and butter. The seed contains 34 per cent protein and 47 per cent fat. A bushel contains more than three times the amount of digestible protein, fat, and ash that is contained in a

[1]Estimate furnished by the North Carolina Experiment Station.

bushel of corn. Soy bean hay contains 2.48 per cent nitrogen, 0.40 per cent phosphoric acid, and 1.32 per cent potash.

It has been found in most of the soy bean growing sections of the South that an acre will produce on the average something like two-thirds to three-fourths as many bushels of soy beans as of corn and the price brought by soy beans has always been from 50 to 100 per cent greater.

During 1915, $9,000,000 worth of oil alone was imported. Cottonseed oil mill owners have been induced, however, partially by the efforts of members of the staff of the North Carolina Experiment Station, to crush soy beans during their otherwise idle season. The few mills in the State which have done this have found a ready market for the oil and meal.

OTHER SOY BEAN DISEASES

Soy beans are very generally observed to be quite free from disease, and no very seriously destructive parasites of this host appear to have been reported in the literature at hand. Of those reported, a detailed study has not been made, except in the case of *Bacillus lathyri* Manns and Taubenhaus (16, 17). The accounts of the other diseases consist of brief fragmentary mycological notes and mention of their place of collection or of their appearance. Since any of them may appear on plants affected with blight or wilt, it is deemed advisable to call attention to the published accounts of these diseases and the appropriate bibliography.

Septoria sojina v. Thümen (on living or declining leaves) (27).
Phyllostica sojaecola Massalongo (18, p. 688).
Aecidium glycines P. Henn. (8, p. 52).
Uromyces sojae (P. Henn.) Sydow (25, p. 429).
Bacillus sp. (on leaves)—Heald (11, 12), Smith (24), and Clinton (4).
Bacillus lathyri Manns and Taubenhaus (on leaves and pods) (17), and Manns (16).
Heterodera radiciola—Scofield (22, p. 9), Gilbert (10, p. 9), Bessey and Byars (2, p. 8). (These authors merely mention the soy bean as a host for this parasite.)
Chlorosis and crinkling (cause?). (Description of the disease in the field.) Clinton (5).
Septoria glycines T. Hemmi (comparison with *S. sojina* above) (13).

A disease due to *Sclerotium rolfsii* is the only one not reported which the writer has observed to seriously injure the crop.

It is not believed that the presence of any of these organisms would lead to confusion in the diagnosis of blight caused by the species of *Fusarium* under consideration.

HISTORY, OCCURRENCE AND IMPORTANCE OF THE DISEASE

No published report of a disease of soy beans caused by any species of *Fusarium* and one account only of attempts to produce a disease of this host with the cowpea wilt organism have been brought to the writer's attention. Orton (19, p. 16-19) conducted these tests at Edisto Island, S. C., in 1900, and at Monetta, S. C. He says (p. 18):

"Eight varieties (soy beans) were tried on ten plats. All proved to be immune to the wilt disease, but none of them was adapted to the local conditions. The growth was very small, the plants averaging from 8 to 14 inches high, tho most of the varieties bore a good crop of seed for such small plants. All suffered from much drought in midsummer and all were badly injured by the root nematode. On examination of the roots a moderate number of bacterial tubercles were found. * * * They (soy beans) were at a considerable disadvantage in this test on account of the late date of planting and the ensuing dry weather."

The varieties tested were Tokio, Buckshot, Yosho, Ito San, Manhattan, Guelph, and Amherst.[1] Orton reported that at Edisto Island the soy bean made a heavy growth, 3 or 4 feet high, and was free from the wilt disease. It may be said that a very considerable proportion of the several varieties of cowpeas grown in adjacent plots succumbed to wilt. The results of these tests accord with the observations of others who have had opportunity to observe these crops when they were grown on soil known to be infested with cowpea wilt.

A limited number of careful observations have therefore been made during 1915 and 1916 to determine whether the *Fusarium* diseases of these two hosts are coextensive in range and thus to furnish evidence of the identity of the two. Two 5-acre fields on widely separated parts of the North Carolina Experiment Station farm, in which cowpeas and soy beans were grown in alternate rows, showed a very considerable proportion of the former host affected, whereas the latter remained entirely free from disease. In other localities of the State, soy beans growing on soil infested with the cowpea-wilt organism have remained disease-free.

Observations differing from these were made in the case of soil brought from another part of the Station farm. When this soil was used to grow soy beans in pots out of doors, it was found to be infested with the soy bean-blight organism, as shown by the development of the disease in 33 of the 80 jars (Plate 95, D and

[1]The names in use for these varieties in 1890 were respectively as follows: Best Green, Early Black, Yoshoka, Rokugatsha, Gosha, Black Round, Green Medium, and Bakaziro.

E). Wilt of cowpeas and blight of soy beans were present on the farm of one of the correspondents previously referred to, at Red Springs, N. C. Many of the soy bean plants in this field were killed and many only stunted, so that a decrease in yield of 60 per cent during the season of 1916 is probably a correct approximation of his loss. Blight of soy beans has also been found to occur at Exum and Belhaven, N. C., and was the cause of considerable loss in both locations. The first of these soils occurs in the eastern edge of the Piedmont and the others in the Coastal Plains section of the State.

Dr. W. H. Tisdale, in conversation with the writer in 1917, said that while at Madison, Wisconsin, his attention was called to diseased soy beans growing in experimental plots which seemed to be infected with a species of *Fusarium*. Upon being shown specimens of the soy bean blight, he stated that the diseased plants looked very similar to those he had previously seen. Dr. G. L. Peltier wrote from the Alabama Experiment Station in the fall of 1917: "It (soy bean blight) was quite common here on the station farm, and I isolated a *Fusarium* from the infected material."

Cowpea-wilt has been found in many localities in parts of the United States. It is also entirely probable, if we judge from the results to be presented subsequently, that the soy bean-blight may appear more or less generally wherever the soil is infested with *Fusarium tracheiphilum*. Records received from the office of

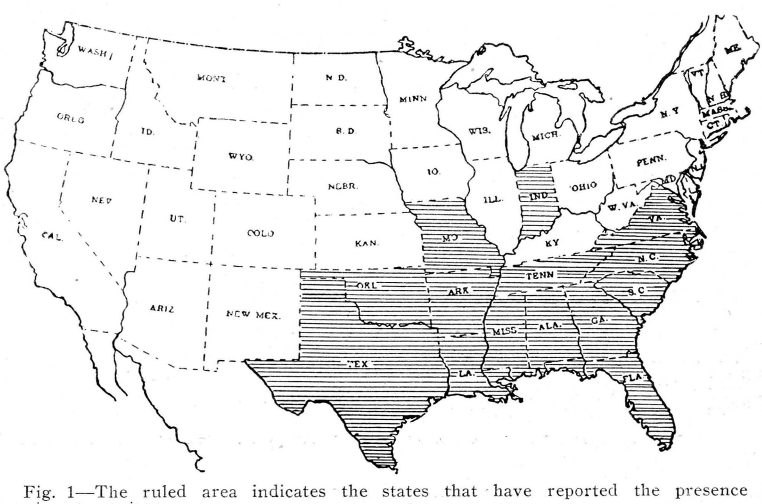

Fig. 1—The ruled area indicates the states that have reported the presence of *Fusarium tracheiphilum*.

Plant Disease Survey show that, up to January 1, 1918, *F. tracheiphilum* has been reported as being productive of losses to cowpeas ranging from 2 to 100 per cent in Indiana, Missouri, Mississippi, Louisiana, Texas, Oklahoma, Georgia, Florida, North Carolina, South Carolina, Virginia, Arkansas, and Tennessee. These states are blackened in the accompanying map (fig. 1).

SYMPTOMS

In 1916, soy beans were planted during the last two weeks in May. This is somewhat later than usual, being due to the late season and a period of drought. When the plants were 4 weeks old, they had attained a height of 2 to 3 dm. and were apparently still free from disease. The disease was first observed on July 25, when the affected plants were about 8 weeks old. Symptoms of the trouble could probably have been found a week or two earlier. Affected plants, all of the same age but varying in height from 2 dm. to 1 meter, were observed on the 25th. The fungus is believed to have stunted these small plants. In no case has the disease been observed to occur on seedlings under field conditions. In sand inoculated with pure cultures, however, a number of seedlings became diseased as described later in this paper.

In 1917, soy beans were planted in the same field on April 26. The weather was cool with considerable rain for the following week or ten days so that on May 27 the majority of the plants had only just broken thru the surface of the soil, some were not yet up, and some were from 10 to 12 cm. in height. Examination of a few of the plants from a number of the rows did not show injury that could be attributed to *Fusarium*. On June 12 some of them were 15 to 25 cm. in height and infected plants were found in many of the rows. On June 26 the plants were 8 weeks old and from 3 to 5 dm. in height. Many were stunted by this time, partly because of the action of *Fusarium* and partly because of nematodes, *Rhizoctonia*, and other causes, so that only by removing suspected plants could it be certain that they were diseased and that *Fusarium* was quite generally present thruout the field. Plants were found without any of the external symptoms described below, except the dwarfing, the xylem areas of which were browned well up the stem. By July 20 the disease was present thruout the field and many plants were in advanced stages of the disease.

Comparing the conditions in 1917 with those of 1916 in the same field, one finds that at the end of 8 weeks the plants were smaller in 1917 but the disease was more general and more advanced in its injury to the individuals.

The contrast in appearance of five healthy and five diseased plants is shown in Figure 3, D. E. The same type of sandy loam soil was used in both jars and the plants in each were grown out of doors under the same conditions. The plants shown in figure E were naturally infected from naturally infested soil and were typical of the diseased plants in 32 other jars of the 80 in the test. A considerable number of the leaves have fallen from the diseased plants, a portion of the petioles persist without leaflets, the plants are dwarfed, and there is no evidence of wilting in any part of the plants. The foliage which persists on these plants is yellow as contrasted with the normal leaf green of healthy plants.

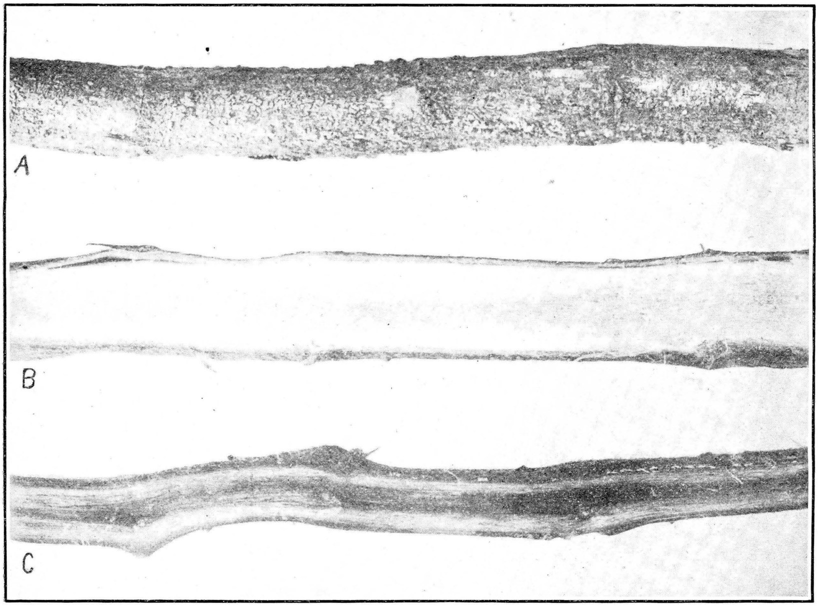

Fig. 2—A. A diseased stem of soy bean, showing the roughened appearance caused by the irregular covering of sporodochia.
B. Interior of healthy (unstained) stem of soy bean.
C. Interior of diseased (discolored) stem of soy bean.

The occasional absence of a definite wilting of the leaves has been noted in other wilt diseases. Orton (19, p. 10), in speaking of the cowpea disease caused by *F. tracheiphilum*, says: "The term 'wilt' is somewhat misleading, as the leaves usually drop off before there is any conspicuous wilting. The name was applied because of its relationship to the wilt of cotton and watermelon, where this symptom is very prominent, and it seemed desirable to retain it for the cowpea disease."

In the case of the soy bean disease, wilting is a less prominent symptom than in cowpeas, and is seldom present at any stage of the disease. The plants, as a rule, drop all of their leaves and die without any evidence of wilting. Wilting has been observed in a very few instances in the field in the case of young plants. The woody nature of the stem and petioles probably accounts for the general absence of wilting in them, and the presence of well-developed mechanical tissues in the leaflets may account for their failure to manifest wilt. The possibility exists, also, that the physiological interaction of parasite and host differs from that exhibited by wilted cotton and watermelons infected with *Fusarium* spp. If potted soy beans which have never been excessively watered are allowed to remain without water they do not wilt but the leaves finally curl as the tissue becomes dry and crisp. Neither do the leaves wilt if the stem is cut to the center.

Fig. 3—D. Soy bean plants grown out of doors in the same type (Cecil) sandy loam soil; D, healthy; E, diseased thru the naturally infested soil.

Instead of applying the name "wilt," therefore, to the soy bean trouble, it is thought desirable to call it "blight." This word describes the most prominent symptom on the foliage.

That plants are often almost indifferent, for some time, to the fungus which may even be abundantly present within it, is shown not only by the absence of wilt but also by the fact that many of the plants mature a reduced crop of beans in spite of the disease. Affected plants in such cases are stunted and mature earlier than healthy plants. In contrast to this, 100 per cent of the cowpeas in rows in different parts of the same field are infected, most of them die before reaching a height of 2 dm., and the scattered plants then remaining may all die before blooming.

Perhaps the most prominent symptom is a browning of the interior of the stems and roots. By the time the lower leaflets or leaves begin to drop, this discoloration is evident well up into the stem and, if necessary, by removing the petioles, infected plants may be noted from the brown color of the bundle scars when no other positive symptom is to be observed. As the disease progresses, the discoloration extends in some cases to the tip of the stem and into the bundles of the leaflets. The tracheal tubes of affected stems when cut obliquely show as brown specks. The relative amount of discoloration in general and the depth of color in affected xylem portions is less in soy beans than in cowpeas. Healthy and diseased stems are shown in Figure 2, B and C, respectively.

The surface of stems of plants in advanced stages of the disease in the field generally have salmon-colored spore masses, sporodochia, thickly and irregularly distributed over them.[1] This character is shown by the roughened appearance of the stem in Figure 2, A. The spore masses are composed of macroconidia of the fungus and are frequently found to occur on plants, the upper leaves of which are still healthy in appearance. Sometimes they are formed only in more advanced stages of the disease.

ETIOLOGY

Sterile seedlings had been grown in test tubes by the method described later in this paper for use as a culture medium to maintain the virulence of the organism. In 1915 the roots of such seedlings, inoculated after the development of the first true leaves, were found in a few days to be penetrated by the fungus thru stomata and epidermis. The roots were mounted directly on slides in a glycerin-eosin water solution and examined under the micro-

[1]Sporodochia on stems of cowpeas are reported by Orton (19, p. 9) to appear after the death of the plants.

scope. Tisdale describes practically the same method in a recent paper (28, p. 576). Under such unfavorable conditions, however, the host was at a disadvantage, and since a number of other species of *Fusarium* penetrated the plants in the same way, no significance is given to the observations.

In order to determine the relation of the organism within the various host tissues, a large number of stained free-hand and microtome sections were made of stems and roots at various stages of development of the disease. A number of attempts to obtain pieces of roots at the time of infection were, however, unsuccessful. In woody stems, in an early stage of the disease, only the xylem tubes nearest the pith were found to contain the fungus filaments. The pith had disappeared in both normal and diseased plants of moderate size. Later, other of the tubes thruout the xylem area were penetrated and had become filled to a large extent with a network of fungous filaments. In still more advanced stages, all of the xylem elements (fig. 4, H) were found to contain the fungus, and in addition the cortical parenchyma was invaded.

Many of the fibrous roots were destroyed, and figure 5 shows how the disease progresses from the smaller root branches into the main root. A and C represent the cortex and pith, respectively, and are, at this stage of the disease, seen to be free from discoloration due to the fungus. B indicates the xylem region, which is discolored in areas beginning at the point of origin of lateral roots and extending mostly upwards. Later the browned areas become continuous and the xylem is found to be in this condition thruout the stem and root (fig. 2, C). New roots form, but often of insufficient number to maintain the life of the plant. In other cases the plants subsist with a reduced supply.

It seems, therefore, that the symptoms produced result not simply from (1) a mechanical clogging or (2) a slow appropriation of food and water by the fungus, as others have previously stated, but that (3) a considerable destruction of the root system, and, perhaps, (4) a reduction of the activity of the protoplasm due to the possible presence of toxins secreted by the fungus, also aid in this.

That other organisms may often facilitate the entrance of *Fusarium* is given special consideration in another part of this paper.

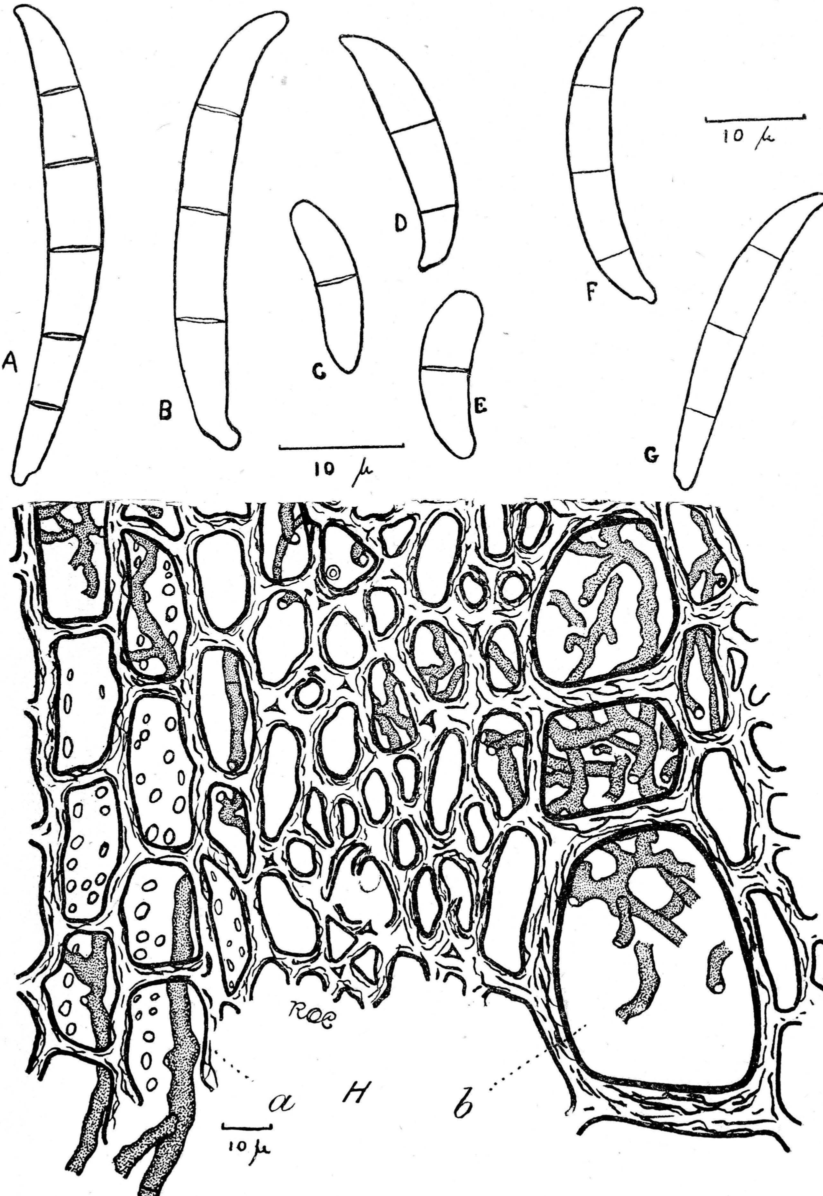

Fig. 4—*A-G*, Types of macroconidia of the species of *Fusarium* on soy bean. *H*, Cross section of the xylem portion of a diseased soy bean stem, showing the invasion of the medullary rays *(a)* and the xylem vessels *(b)* by mycelia of the species of *Fusarium* on soy bean.

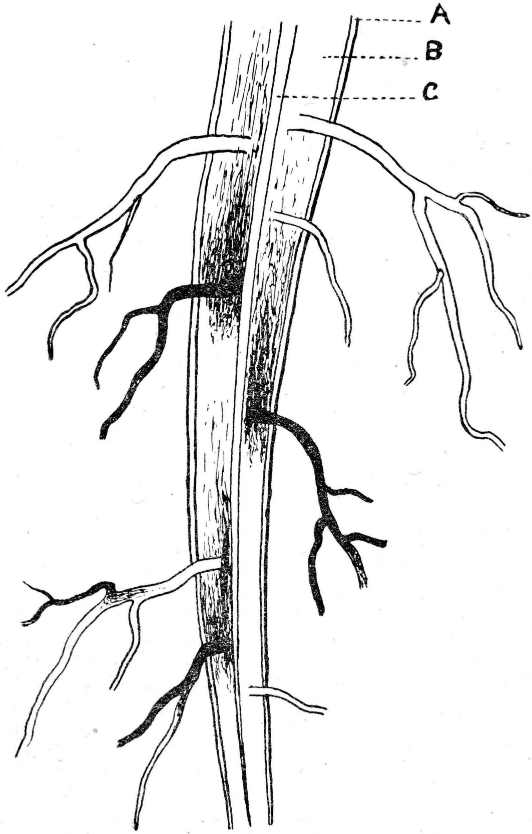

Fig 5—Diagrammatic drawing of a longisection of the main root of the soy bean to show the entrance of the fungus from the lateral roots. A and C indicate uninvaded pith and cortex, respectively. B represents the discolored xylem in an early stage of the disease.

COMPARISON OF THE SOY BEAN SPECIES OF FUSARIUM WITH OTHER WILT-PRODUCING SPECIES OF THE GENUS

SOURCE OF CULTURES AND METHODS OF ISOLATION

Isolations were made from the interior of stems of freshly wilted soy bean and cowpea plants. The stems were first thoroly washed in water and allowed to remain wrapped in cotton moistened with 0.1 per cent solution of mercuric chlorid for 15 minutes. They were then split open so that the diseased interior was exposed. Fragments of diseased tissue were removed with a sterile scalpel and transferred to cooled poured plates of string-bean agar (8 c.c. per plate), to each of which four drops of 20 per cent lactic acid had been added. After several days, a microscopic examination was made of the conidia and mycelium to determine whether other organisms were present. Eight transfers to test-tube slants were made from the margin of several plantings and kept for comparison and for indications of contamination. It may be noted that a large percentage of pure cultures was obtained by this method. From the cultures that were pure, single-spore cultures were obtained according to the method described by Sherbakoff (23, pp. 102-103; p. 104, footnote 8). Stock cultures were made from these single-spore cultures and repeatedly repoured to protect from subsequent contamination.

Several species of *Fusarium* were secured, in order to compare them with the *Fusarium* sp. from the soy bean and the one from the cowpea, isolated as described above. The following species, subcultures from Wollenweber's authentic cultures, were obtained thru the courtesy of Mr. C. W. Carpenter, of the Bureau of Plant Industry: *Fusarium oxysporum* (Schlecht.), *F. vasinfectum* (Atk.), *F. lycopersici* Sacc., *F. niveum* Smith (members of the section *Elegans)*, and *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw. (1, pp. 115-118), (section *Discolor)*.

These species were studied in culture, in order to determine their morphological and cultural characters, since such a study is considered of primary importance in their differentiation. The species mentioned were chosen because all except one belong to the section *Elegans*, the section which contains the known wilt-producing species, and because, according to Wollenweber, they are the most difficult to separate by this method. *F. conglutinans* Wollenw., *F. redolens* Wollenw., and *F. orthoceras* App. and Wollenw., of the same section are included in the comparisons. They are so different from the others, as indicated by the original descriptions, that the writer soon realized that there was little probability of confusing them with the soy bean strain. Wollenweber (31, 32) and Sherbakoff (23) have described other species and varieties of the section *Elegans*, which are not, however, included in this study, because they occur on hosts widely separated genetically from the soy bean[1] and because the authors have not had opportunity to make a sufficient number of infection experiments to establish them as wilt producers.

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[1]Wollenweber, H. W. (31, p. 37) says, "The parasite from one host has not been found on living organs of another host. In pure culture the parasite from one host did not cause wilt in any other host as a result of inoculation experiments."

CULTURE MEDIA AND THEIR VARIOUS EFFECTS ON SPECIES OF FUSARIUM

In making a cultural study of these fungi much care was taken to follow the suggestions of Appel and Wollenweber (1), Wollenweber (31, 32), and Sherbakoff (23), in order to determine what criteria to employ in judging normal growth characters. It is generally believed that standardization of cultural methods is highly essential in the comparative study of so difficult a group of fungi.

The writer has kept the soy bean and the cowpea strains under constant observation for three years and other strains for a part of this time, on various kinds of "natural and artificial media" and under widely variable physical conditions. He is therefore familiar with the possible variability of members of this genus.

Since a large number of the media used did not prove to be of special diagnostic value, they are not discussed here. Among the media most commonly employed and serving some particular purpose were oat, potato, and string-bean hard agars (3 per cent agar), which, because of the paucity of moisture (23, p. 106), give all forms of fructification with "normal" spores. Five to 10 per cent of dextrose was added to agars to favor the production of pigment. The addition of this sugar, however, favored the development of mycelium at the expense of macroconidia, and when from 8 to 10 per cent was added these spores were often absent. Growth on steamed rice in test tubes from weighed quantities of rice and measured amounts of water to obtain uniformity also results in the formation of pigment and sometimes an odor that is typical for certain related species of *Fusarium*. Herbaceous and woody stems, string-bean pods, and potato plugs give the best development of sporodochia and pionnotes.[1] Potato plugs also serve for the proper development of sclerotia and colors, both of which may be reduced or absent from stem plugs when there is a minimum development of mycelium.

According to Wollenweber (31, p. 37), virulence is commonly maintained on stem plugs. Living sterile soy bean and cowpea seedlings grown in 6-inch test tubes were also used and are thought to be a better medium for maintaining virulence in the strains from the respective hosts.

In order to obtain sterile seedlings for this purpose the seeds were first washed for 5 minutes in tepid water and were then placed in concentrated sulphuric acid for 20 minutes. Formalin, mercuric chlorid, both in aqueous and alcoholic solution, and

[1]For a discussion of these terms, see Wollenweber (31, p. 24).

other disinfectants were employed with much less success. After washing off the acid in three or four changes of sterile water, the seeds were transferred into sterilized moist chambers in the bottoms of which several layers of moist filter paper had been placed. Germinated seeds on which there was no evidence of contamination after a day or two were transferred to sterile test tubes[1] the bottom of each of which contained a wad of moistened filter paper.[2] If, during germination or transfer, contamination occurs, it generally becomes evident on the seedlings or white paper, especially if the seedlings are set aside until they have grown to a height of 3 or 4 inches.[3]

More recently, however, such precaution to maintain virulence was found to be unnecessary because the age of cultures seemed to have little effect upon their virulence. Old cultures gave as high a percentage of infection, if grown on proper media and transferred frequently, as they did when first isolated.

METHODS OF STUDY AND PRESENTATION

All transfers of different strains in a set for comparison were made to a certain medium on the same day and to additional media to provide the necessary cultural characters. When species were compared, they were always of the same age and were grown on the same medium. As many comparisons could be made on the same day as there were species and kinds of media in the set. If sufficient data had not been obtained, if certain cultures were abnormal, or if other species or media were to be used, new sets were prepared of all of the species using the desired media and comparisons were again made thruout the series.

Cultural differences also arise as a result of the employment of spores or a bit of mycelium in inoculation. In the former case the young cultures quickly produce spores with a scant mycelial growth, while in the latter the mycelial growth is abundant and there is a paucity of spores. For this reason spores from sporodochia, when present, were used, and in all cases, in so far as was possible, the same kind of inoculum was transferred for all cultures of a set. When the production of spores becomes subnormal, as it often does in cultures, considerable time and patience may be required to bring the strain back to a "Normkultur." This was

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[1]For making this last transfer, dip the ends of long tweezers into 95 per cent alcohol and ignite in the flame. This sterilizes instruments, burns off the excess of alcohol, and leaves them dry and cool enough for immediate use.

[2]The use of agar as a substratum for this purpose (Garman and Didlake, 9), and Sphagnum moss, did not prove to be satisfactory. Soil, too, has a disadvantage in that it does not show the contaminations as readily as filter paper or agar.

[3]An oat sprouter with glass front, heated by a kerosene lamp and costing about $10, makes a good light incubator for such purposes when the greenhouse is not conveniently located or the temperature suitable. This sprouter is unsuited, of course, to cultures or material requiring a constant temperature.

accomplished by transferring a small portion of mycelium to a variety of media until a medium was found on which spores were again obtained.

All cultures were kept in the laboratory at room temperature, 12° to 26° C., and in diffused daylight, so that they were subjected alike to any change of environmental conditions.

In all cases 10 cultures of a species were made on each medium. Different forms of fructification which normally appear on a certain medium may not do so in every tube. For example, in a species in which sporodochia are not abundant, they may perhaps form on only 2 or 3 of the 10 stem plugs; or if the form produces green sclerotia, they may develop on not more than 5 of the 10 potato plugs. In some instances as many as 8 to 10 sets of 10 tubes each of a particular species were made.

In making the microscopical examination, note was taken of the size, septation, abundance, and type of conidia (fig. 1, A-G), chlamydospores, and conidiophores. In measuring spores, several fields were first examined to fix in mind the prevailing type and an average of 10 or more of these typical spores was made. Careful note was taken also of extreme types.

In the macroscopic study of the cultures, the nature of the stromata, the pionnotes and sporodochia, the character of the aerial mycelium, the color of spore masses, aerial and submerged mycelium, and substratum, and the production of sclerotia were considered.

RESULTS OF THE COMPARISON OF THE SOY BEAN FUNGUS WITH OTHER MEMBERS OF THE SECTION ELEGANS

The first sets of parallel cultures were intended to serve in the separation of any or all of the species of *Fusarium* causing wilt from the soy bean fungus. *F. discolor*, var. *sulphureum*, *F. oxysporum*, *F. vasinfectum*, *F. lycopersici*, *F. niveum*, *F. tracheiphilum*, and *Fusarium* sp. from soy bean were therefore grown on the following media, several sets of 10 cultures of each species being used on each medium: Potato plugs, steamed rice, cotton stems, potato hard agar, and string-bean hard agar. The cultures were examined when 8, 15, 19, 30, and 50 days old. The results are noted in Table 1. Only those characters are recorded that are necessary for the separation of the species.

TABLE 1.—*Characters which separate a number of the wilt-producing species of* Fusarium *from* F. tracheiphilum *and the soy bean fungus.*

Species	Sclerotia	Sporodochia	Pionnotes	Chlamydospores
F. discolor........	None	Numerous	Perfect	Intercalary; no measurements.
F. vasinfectum....	Green and flesh-colored	do	do	Intercalary and terminal; no measurements.
F. oxysporum.....	do	Few	Reduced	Intercalary and terminal; 6 to 12 μ.
F. lycopersici.....	Flesh-colored	Numerous	Perfect	Intercalary and terminal; no measurements.
F. niveum........	Large green	do	Reduced	Same as for *F. lycopersici.*
F. tracheiphilum...	Green and flesh-colored	Few	None	Intercalary and terminal; 6 to 12 μ.
Fusarium sp. on soy bean........	Mostly green; some flesh-colored	do	do	Same as for *F. tracheiphilum.*

TABLE 1 (Continued).—*Characters which separate a number of the wilt-producing species of* Fusarium *from* F. tracheiphilum *and the soy bean fungus.*

Species	Macroconidia		Odor
	Size of 3-septate	Type	
F. discolor........	No data	*Discolor;* mostly 5-septate	None.
F. vasinfectum.....	Same as in *F. oxysporum*	*Elegans;* mostly 3-septate	Strong lilac on rice.
F. oxysporum.....	28.7 to 35.6 by 3.6 to 4.1 μ	do	Often none, sometimes scant lilac.
F. lycopersici......	Abnormal	do	None.
F. niveum.........	Abnormal; (original description gives larger than *F. oxysporum*).	do	do
F. tracheiphilum...	23.6 to 41.0 by 3.9 to 4.1 μ	do	do
Fusarium sp. on soy bean........	24.6 to 35.8 by 2.89 to 4.1 μ	do	do

From the data in Table 1 it is important to observe that *F. tracheiphilum* and the species of *Fusarium* on soy bean belong to the section *Elegans*, as established by Appel and Wollenweber (1) and modified by Wollenweber (31) in a subsequent study. They are themselves very similar in cultural characters, but can be quite sharply separated from the other species included in the tabulation. When the characters of the species of *Fusarium* on the cowpea and soy bean noted in this table are compared with those in the original descriptions of certain other members of the section *Elegans*—namely, *F. redolens*, *F. orthoceras*, and *F. conglutinans*—there is plainly no chance of their confusion. *F. redolens* (31) produces no blue sclerotia, and its conidial masses are brownish white; *F. orthoceras* (25) possesses neither sclerotia, sporodochia, nor pionnotes; and *F. conglutinans* (31) is distinguished because of the absence of sclerotia, sporodochia, or pionnotes, and the typical wine-red to purple colors of the section.

MORPHOLOGICAL AND CULTURAL COMPARISON OF THE FUSARIUM SP. ON SOY BEAN WITH F. TRACHEIPHILUM

Since the studies summarized in Table 1 do not succeed in distinguishing the species of *Fusarium* on soy bean and cowpea, a more extensive cultural study of these two fungi was made. For this purpose three series of cultures were grown, and the results have been summarized in Table 2. Each series contained 10 cultures of each fungus on stem plugs, potato plugs, steamed rice, standard nutrient agar (1.8 per cent agar, 1.0 per cent acid), string-bean hard glucose agar (3 per cent agar, 1.0 per cent acid, and 10 per cent glucose), and oat hard agar (3 per cent agar and 1.0 per cent acid). The cultures were examined when they were 8, 15, 30, 50, and 75 days old.

TABLE 2.—*A morphological comparison of the species of* Fusarium *on soy bean and cowpea.*
Fusarium *sp. on soy bean.*

Medium	Macro-conidia	Sporodochia	Sclerotia	Color of mycelium	Character of mycelium
Standard nutrient agar	No measurements	Salmon-colored	None	White	Mostly aerial and floccose, becoming appressed in old age.
String-bean agar......	do	Salmon-colored; generally present	Green	do	do
Oat, hard glucose agar......	26.6 to 38.6 by 3.69 to 4.92 μ, 50 days old	Flesh-colored	Dark-green	Mostly lilac; some dark purple	Cottony.
Steamed rice	....	....	....	Reds, pinks, lilacs, purples	....
Potato plugs	Normal spores absent	Salmon-colored; generally present on sclerotia	Dark-green	Green, near sclerotia	Floccose.
Stem plugs.	22.5 to 43.6 by 2.87 to 4.11 μ, 14 days old	Salmon-colored; small	Green, very small; numerous	White, sometimes green near sclerotia	Floccose; scant.

TABLE 2 (Continued).—*A morphological comparison of the species of* Fusarium *on soy bean and cowpea.*
F. tracheiphilum.

Medium	Macro-conidia	Sporodochia	Sclerotia	Color of mycelium	Character of mycelium
Standard nutrient agar......	....	None	Flesh-colored	White	Mostly submerged or appressed
String-bean agar......	No measurements	Salmon-colored; few	Mostly flesh-colored; some green	do	do
Oat hard glucose agar......	22.5 to 36.9 by 3.8 to 4.42 μ 50 days old	Flesh-colored	Dark-green and flesh-colored	Mostly dark purple; some lilac	Cottony to matted and appressed
Steamed rice	....	....	....	Pinks, reds, lilacs, purples	....
Potato plugs	24.6 to 36.9 by 3.28 to 4.42 μ 19 days old	Salmon-colored; often on sclerotia	Flesh-colored; often none	Pinks, lilacs, greens	Mostly appressed
Stem plugs.	No measurements	Salmon-colored; small; sometimes none	Green; very small; numerous	White; sometimes green, near sclerotia	Appressed; good growth.

No mention is made in Table 2 of pionnotes or odors, as none was produced in any of the cultures. Only color-production was noted on steamed rice. The macroconidia of both strains show a wide variation both in size and in shape, but these differences can properly be included in the range of variation. The normal macroconidia of the soy bean (fig. 4, A-G) and cowpea strains are indistinguishable. The chlamydospores of either strain are terminal or intercalary in or on vegetative filaments and average 6 to 10.25 μ in diameter. The conidiophores are verticillately branched when normal. Sporodochia, altho sometimes flesh-colored, are normally salmon-colored. They are not always present on all media but are formed by each strain either on sclerotia or on mycelia as stromatal bases. Green sclerotia are normally present in both strains. There appear to be some differences in colors produced in substrata, altho not very consistent ones, a difference in the character of mycelium until advanced ages of the cultures and generally, but not always, an absence of flesh-colored sclerotia in the soy bean fungus. These differences, however, are not believed to be of sufficient importance to warrant regarding the soy bean strain as a distinct species or variety.

In addition to the media employed in Table 2, potato hard agar, cornmeal plugs, and string-bean pods were used, but they showed no additional characters of value.

Perithecia have never been observed on the diseased stems; neither have they been obtained in cultures from the surface spores, nor from the diseased internal tissues. In fact, the cultural differences between the *Fusarium* sp. on soy bean and *Neocosmospora* spp. are as striking as between *Neocosmospora* spp. and the several species of *Fusarium* causing wilt studied by Higgins (14) and Butler (3).

INOCULATION EXPERIMENTS

From the foregoing morphological and cultural studies, it is evident that the species of *Fusarium* on soy bean is not distinguished from *F. tracheiphilum* by any well-defined differences. Since the possibility existed that they might be separated by biological differences, reciprocal inoculation studies were undertaken to secure additional evidence of their identity.[1]

Plants were therefore grown in pots and flats in the greenhouse and in plots in the field for use in inoculations. The soil used in the pots and flats was a fine, compact, sandy loam, except in the case of one experiment, and was taken from a field in which diseases of cowpeas and soy beans caused by *Fusarium* spp. had never been observed. In certain of these tests, as an added precaution, the soil was partially sterilized by the use of a 2 per cent solution of formaldehyde. The seed were also sterilized in certain experiments by immersion for 15 minutes in commercial sulphuric acid. Since uninoculated plants remained free from disease when these precautions were not employed, their use was discontinued in subsequent tests, unless otherwise stated.

The pots and flats were of sufficient size to permit the plants to grow to maturity.

In determining the percentage of diseased plants, count was made only of those in which it was possible to find discoloration and invasion of the xylem tissues. In case of doubt in this microscopic examination, planted plates were made from the tissues and the subsequent growths studied.

The varieties of soy beans and cowpeas planted for the cross-inoculation experiments were known to be subject in the field to the species of *Fusarium* on soy bean and cowpea, respectively.

[1]Wollenweber (31, p. 37) says that a consideration of the biological characters is of secondary importance in the determination of species.

EXPERIMENT I.—Twenty-five North Carolina Black cowpea and 25 Mammoth Yellow soy bean seedlings, growing in each of two flats in the greenhouse, were each inoculated when from 10 to 15 cm. high with spores from sporodochia and with mycelium by introducing the material into incisions in the stems at 2 to 4 cm. below the surface of the soil. All of the plants in one flat were inoculated with the soy bean strain of *Fusarium* and all of those in the other with the cowpea strain. Checks and all inoculated plants except two cowpeas inoculated with the soy bean strain and one with the cowpea strain remained healthy. The test was repeated, using freshly isolated strains of both organisms; and, since all but one of the plants remained healthy, this method of inoculation was discarded.

EXPERIMENT II.—In this experiment the soil in two flats, A and B, in the greenhouse was inoculated with pure cultures of *Fusarium* spp. on cowpea and soy bean, respectively. These cultures were then incorporated in the upper 4 inches of soil.

In this and succeeding experiments the organisms had been grown on pieces of moistened, sterilized cowpea stems until numerous sporodochia had formed. On April 12, 1916, 20 North Carolina Black cowpeas and 20 Mammoth Yellow soy beans were planted in each flat. A third flat, containing uninoculated soil, was planted as a check.

By June 4 a cowpea plant on Flat B was noted to be diseased. Others had been observed to be affected by June 15, when all the plants were removed and examined. The results are presented in Table 3.

TABLE 3.—*Results of growing soy beans and cowpeas in artificially inoculated soil.*

Flat	Organism	Host	Total number of plants	Diseased plants	
				No.	Percentage
A	*F. tracheiphilum* on cowpea. .	Cowpeas. . . .	20	6	30
		Soy beans. . .	20	3	15
B	*Fusarium* sp. on soy bean. . .	Cowpeas. . . .	20	10	50
		Soy beans. . .	20	7	35
C	None (control).	Cowpeas. . . .	20	0	0
		Soy beans. . .	20	0	0

EXPERIMENT III (Series 1).—Since the percentage of diseased plants in Experiment II is relatively small, the test was repeated, using another strain of each organism and Clay cowpeas instead of North Carolina Black variety. The roots of each plant in this test were injured during the process of cultivation. The period of growth of these plants extended from June 29 to September 1, at which date the plants were fully matured. The results of this series are recorded in Table 4 (a).

EXPERIMENT III (Series 2).—The test in series 1 was duplicated between September 7 and November 20, with no resultant increase in the percentage of infections.

EXPERIMENT III (Series 3).—This test was in duplication of the other series in this experiment except that the soil consisted of a mixture of six parts of coarse sand, one part of fine sandy loam, and one part of stable manure. The results obtained between September 7 and November 20 are included in Table 4 (b), because they show considerable increase in the percentage of infection even tho the cultures used were transfers from cultures nearly 3 months old.

TABLE 4 (a) and (b).—*Results of growing soy beans and cowpeas in artificially inoculated soil, the plants having been wounded below the surface of the soil.*

(a)

Flat	Organism	Host	Total number of plants	Diseased plants	
				No.	Percentage
D	*F. tracheiphilum* on cowpea..	Cowpeas....	20	3	15
		Soy beans...	20	3	15
E	*Fusarium* sp. on soy bean...	Cowpeas....	20	6	30
		Soy beans...	20	5	25
F	None (control)............	Cowpeas....	20	0	0
		Soy beans...	20	0	0

(b)

Flat	Organism	Host	Total number of plants	Diseased plants	
				No.	Percentage
G	*F. tracheiphilum* on cowpea..	Cowpeas...	20	16	80
		Soy beans...	20	12	60
H	*Fusarium* sp. on soy bean...	Cowpeas...	20	13	65
		Soy beans...	20	12	60
J	None (control)............	Cowpeas....	20	0	0
		Soy beans...	20	0	0

EXPERIMENT IV.—Since it was thought that the strains of *Fusarium* on soy bean had to a degree lost their virulence by growth in culture, soy bean stems bearing an abundance of sporodochia were macerated and mixed with the soil in two flats. Seed of the Mammoth Yellow variety were planted on May 25. When the experiment was concluded, August 10, only 8 of the 80 soy bean plants in these two flats were found to be infected.

EXPERIMENT V.—This experiment was designed to confirm the results of inoculations in the greenhouse by inoculations under partially controlled field conditions. Four small plots (Nos. 26, 27, 28, and 29) on wilt-free soil of the station farm were inoculated with the *F. tracheiphilum* from soy bean; two others (Nos. 59 and 60) with this organism from cowpea, and two (100 and 101) were left untreated as controls. Thirty cowpeas and thirty soy beans were planted in each plot on June 10, and the final results noted in Table 5 were obtained on September 4.

TABLE 5.—*Results of cross-inoculations in the field.*

Plot No.	Organism	Host	Total number of plants	Diseased plants	
				No.	Percentage
26	*Fusarium* sp. on soy bean	Clay cowpeas Haberlandt soy beans	30 30	17 4	56.6 13.3
27	do	Clay cowpeas Tokio soy beans	30 30	10 0	33.3 0.0
28 28	do do	Clay cowpeas Mammoth yellow soy beans	30 30	10 8	33.3 26.6
29	do	Clay cowpeas Tar Heel Black soy beans	30 30	15 3	50.0 10.0
59	*Fusarium* sp. on cowpea	Clay cowpeas Tokio soy beans	30 30	26 6	86.6 20.0
60 60	do do	Clay cowpeas Mammoth Yellow soy beans	30 30	17 6	56.6 20.0
100	None (control)	Clay cowpeas Mammoth Yellow soy beans	30 30	0 0	0.0 0.0
101	do	Clay cowpeas Mammoth Yellow soy beans	30 30	0 0	0.0 0.0

EXPERIMENT VI.—On May 25, 1916, two 100-foot rows of each of the soy bean varieties Tokio, Haberlandt, Mammoth

Yellow, Medium Yellow, and Virginia were planted in a field which produced a large percentage of wilt in cowpeas in 1914. Two rows of cowpeas were planted in the same plot. By September 1, when all the plants had fully matured, a small percentage of wilted cowpeas had been noted; but no blighted soy beans were found.

Similar data were obtained from observations on cowpeas and soy beans grown in the experimental plot devoted to plant breeding. In this 4-acre plot, three or four rows of soy beans were alternated with three or four rows of cowpeas thruout the field. Some wilt occurred in practically every row of cowpeas in the plot, but careful examinations during the season failed to reveal a single soy bean blighted with *Fusarium* sp. among 17 standard varieties and 50 other unnamed selections.

DISCUSSION OF THE RESULTS OF INOCULATION EXPERIMENTS

In inoculation experiments soy beans and cowpeas, from the time of germination until 4 or 5 days after their appearance above the surface of inoculated soil, were often killed by an organism which invaded the cotyledons and the growing tips. This was especially true if nearly pure sand was used instead of soil. It was not definitely proved that *Fusarium* was the cause of this condition, however, and no record of these cases was made in the results given above.

In the results recorded, the cowpeas after 4 to 6 weeks were generally invaded well up into the stem and the plants were often killed after a period in which they showed, except for sporodochia, the striking symptoms commonly known to appear in connection with this disease in the field. On the other hand, the affected soy beans have shown symptoms only on the roots. Some of these were found to have typical discoloration in the xylem areas but the root systems were never invaded to the extent that the plants showed the effects above the ground. A lack of vigor was apparent in many soy beans but it was uncertain without an examination of the roots whether this was due to the action of the organism or to more or less unfavorable conditions for plant growth.

That reciprocal inoculations were successful in a number of cases can be seen from the results of certain of the above experiments in which plants of both hosts became infected by both strains of *Fusarium*. Certain other attempts were made, however, in which all soy beans remained free from disease. Contrary to this, the cowpeas in the same flats always showed at least several plants typically diseased, regardless of whether they were growing in soil that had been inoculated with the cowpea or with the soy bean strain of *Fusarium*.

EXPERIMENTS ON THE RELATION OF VARIOUS SOIL FACTORS TO INFECTION OF SOY BEANS BY F. TRACHEIPHILUM

THE INFLUENCE OF SOIL TYPES

Since artificially inoculated soy beans have not always developed the disease, nor developed it to the same degree of severity that cowpeas have under the same conditions, nor as severely as naturally infected soy beans in the field, a number of experiments were planned to determine the factor or factors which seemed to be responsible for the limitations. At the outset possibly all of the factors of the air and soil that influence the health of plants might be concerned. The effects of certain of these have been under consideration in the following experiments in which, with the exception of that on Norfolk soils, all of the plants were subjected alike to those factors which it was impossible to control. In this way evidence was obtained as to the effect produced by certain factors by varying one, under control, in each experiment.

The relation of conditions above the ground, such as temperature, humidity, and light, to the susceptibility of plants to parasitic diseases is often an important one, but a study of them in connection with the disease at hand has not yet been made because a more direct relation probably exists between soil factors and infection. In consideration of the soil, attention has first been given to the effect of variation in texture which is limited by the size of soil particles and by organic and inorganic materials which further influence such factors as temperature and water holding capacity. The United States Bureau of Soils has divided soils into types, classes, and series.

Soil class—"Soil types, which constitute the units of soil classification, may be grouped in different ways. As soils are made up of particles of different sizes, they may be grouped according to the relative proportions of the particles of different sizes which they contain. This grouping is known as soil class and is based on texture." (30, p. 16.)

Soil series—"It has been found that in many parts of the United States a given set of soil classes are so evidently related thru source of material, method of formation, topographic position and coloration that different types constitute merely a gradation in the texture of an otherwise uniform material. Soils of different classes that are thus related constitute a series. A complete soil series consists of material similar in many other characteristics but grading in texture from stone and gravel on the one hand, thru sand and loams, to a heavy clay on the other." (30, p. 19.)

In North Carolina, *Fusarium tracheiphilum* occurs mostly in the Coastal Plains or eastern one-third of the state and in this section the Norfolk soils series is the most important one, not only because of its percentage of area but also because of its agricultural value. These soils are characterized by the light gray to grayish-yellow color of the surface soils and by the yellow color and friable structure of the subsoils. They occupy nearly level to rolling uplands thruout the Atlantic and Gulf Coastal Plains, and have been derived mainly from Piedmont and Appalachian material.

The writer has observed soy bean blight in the field on sand, fine sand, sandy loam, and fine sandy loam soils, but not on those with a higher percentage of the finer particles such as silt, silt loam, clay loam, or clay.[1] Inoculations in the greenhouse thru mixed soils to which a large amount of coarse sand had been added also resulted in a higher percentage of infection. Wollenweber (31, p. 46) concluded, from observations made during his inoculation experiments, that light sandy soils[2] favored the development of *Fusarium* spp. inhabiting the soil and causing wilt diseases.

An experiment was conducted in which soy beans were grown in eleven types of soil of the Norfolk series which were collected with considerable care from typical localities in the Coastal Plains with the assistance of Mr. C. C. Logan of the North Carolina Station who has had considerable experience in making soil surveys. That there are no well-defined limits to individual types, and that different survey parties often disagree in classification and limits of areas, was kept constantly in mind. Consequently soil maps were used only to assist in the general location of several examples of a type from which an average could be selected. Furthermore, as nearly as it was possible, soils were selected which contained a normal amount of humus for the type and to which fertilizers had not been added since the previous crop had been harvested but to which they had been added in the form of complete commercial fertilizers for the benefit of that crop. So far as known neither soy beans nor cowpeas had ever been grown on these soils.

Each type was obtained in quantities of 600 pounds. Two hundred pounds of the first six inches (surface), 200 of the second

[1]The soil referred to previously by the writer (6, p. 424) as a clay was instead Cecil sandy loam.

[2]By this term it is supposed that he referred to soils with a larger percentage of coarse than of fine particles.

six (subsurface), and 200 of the third six (subsoil) were placed in separate sacks, transported to the station at West Raleigh, and replaced at their original depths, in wooden frames sunk to the level of the surrounding soil. These types were arranged in a sequence according to the increase in the percentage of fine particles. The types used were as follows and were arranged in the order given: Coarse sand, sand, fine sand, very fine sand, coarse sandy loam, sandy loam, fine sandy loam, very fine sandy loam, loam, silt loam and clay (30).[1] When arranged in this way, variation in the sequence from type to type was evident. They were each artificially infected with *F. tracheiphilum* from soy bean and the nematode *(Heterodera radicicola)*. A liberal number of nematode galls from the roots of plants not susceptible to *Fusarium* and cultures of the fungus on cut stems were incorporated into the upper six inches of soil. Soil acidity tests indicated that the different lots ranged from approximately neutral to slightly acid.

At best it was hoped to obtain, quite approximately, a series each part of which should vary from the others in its mechanical analysis but as little as possible in all other respects, and from any variations that might appear in the number and severity of resulting infection to get some indication of the cause for the same. If the results should warrant it, a careful physical and chemical analysis could be made from samples of each type that had been set aside for this purpose before the seed were planted.

Soy beans and cowpeas were planted on July 3, 1917, and the cowpeas were removed after the disease occurred on several of them in each flat. The soy beans were harvested on September 26, at which time the plants were mature. The results are presented in Table 6.

[1]For the mechanical analysis of these types see 30, pp. 46-54, and soil surveys of various regions containing these types.

TABLE 6.—*Influence of soil types on percentage of infection by* Fusarium *spp. in the presence of the nematode* (Heterodera radicicola).

Type	Total number of plants	Number with nematodes	Per cent with nematodes	Number with *Fusarium*
Norfolk coarse sand.........	25	4	16	0
Norfolk sand...............	30	8	26.6	0
Norfolk fine sand...........	34	8	23.5	0
Norfolk very fine sand.......	34	6	17.6	0
Norfolk coarse sandy loam...	33	5	15.1	0
Norfolk sandy loam.........	33	8	24.2	0
Norfolk fine sandy loam.....	31	4	12.9	0
Norfolk very fine sandy loam.	25	7	28.0	0
Norfolk loam...............	15	12	80.0	0
Norfolk silk loam...........	17	1	5.8	0
Norfolk clay................	30	0	0.0	0

None of the plants were infected by the fungus and consequently the original purpose, for which the experiment was planned, failed. The results, however, are included since they furnish some evidence that previous failures of a similar nature were not due primarily to the use of soils of unsuitable type, water holding capacity, or humus content. The percentage of infection of cowpeas, however, seems to be increased when growing in inoculated soils containing an excessive amount of humus.

THE INFLUENCE OF ACIDITY AND ALKALINITY

No definite experiments have yet been undertaken to determine the influence of soil acidity on the development of the disease, but field observations indicate that it is not a factor of primary importance. They have shown that the cowpea wilt is destructive on soils varying considerably along the plus and minus scale of acidity.

A similar conclusion can be drawn from the following cultural studies. Two strains of *F. tracheiphilum*, one from cowpea and one from soy bean, were grown on a culture medium whose reaction was varied from +40 to —40 (Fuller's scale) by the addition of standard solutions of $NaOH$ and HCl to a standard nutrient agar, the original acidity of which was +8. Phenolphthalein was used as an indicator. The results presented in Table 7 do not include the measurements of colonies on cultures the acidities of which were above +25, for the reason that the medium was liquefied by so large an amount of acid. Fairly good growth was obtained thereon, however, in 4 or 5 days.

TABLE 7.—*Growth of* F. tracheiphilum *at varying degrees of acidity.*

Age of colony in hours	Diameter of colonies in mm. at various degrees of acidity[2]													
	+25	+20	+15	+10	+5	0	—5	—10	—15	—20	—25	—30	—35	—40
24....	9	10	9	9	9	9	10	10	10	8	8	8	....	....
42....	26	25	22	22	24	21	21	20	18	19	18	18	....	....
68....	43	43	41	42	37	33	32	30	29	27	24	24	22	22
92....	55	55	....	....	48	43	40	37[1]	34	36	36	36	34	34
115....	66	64	66	66	....	....	...	44[1]	42	....	....	....	42	42
140....	77	78	74	72	63[1]	60	60	53	50	53	52	53	48	49
165....	83	83	80	80	69	67[1]	66	60	67	63	63	62	62	62

The strain from soy bean

Age of colony in hours	Diameter of colonies in mm. at various degrees of acidity[2]													
	+25	+20	+15	+10	+5	0	—5	—10	—15	—20	—25	—30	—35	—40
24....	12	9	7	7.5	9.5	9	9	7	6	8	8	8	....	....
42....	23	24	18	20	23	22	21	19	18	17	17	18	....	....
68....	40	41	38	39	35	34	33	32	31	28	28	28	25	25
92....	53	53	....	....	47	46	44	39	38	39	39	40	36	35
115....	60	62	62	64	....	....	...	47	47	....	....	....	40	39
140....	69	73	70	72	66	64	10	55	55	55	51	57	50	47
165....	83	83	79	80	76	75	68	68	63	67	68	67	60	57

The strain from cowpea

[1]Contaminations.

[2]The figures are approximate to within 2 or 3 mm., and are an average of the measurements from ten plates.

From an examination of the table as a whole it is evident in the case of both strains of the organism that there was better growth in the presence of various amounts of acid than in the presence of alkali, but that even on a strongly alkaline substratum the colonies required only 2 or 3 days longer in which to extend themselves over the entire surface of the medium in a petri dish.

Edgerton (7, p. 12) reports field tests with *F. lycopersici* in which tomato wilt was only temporarily checked by the application of lime. He says, "The heavy application of lime (10 tons per acre) decreased the wilt and the plants produced a larger yield of tomatoes. * * * As the season advanced, however, most of the plants in the treated plot also died with the disease."

THE INFLUENCE OF THE NEMATODE (HETERODERA RADICICOLA)

As noted in the above experiment with the eleven types of the Norfolk series of soils, nematodes had been introduced with the *Fusarium* cultures into each flat. This experiment differed from others in which nematodes were present in that the conditions were those of the field under slight modification. The plants were grown to maturity and only a few nematodes were present on the plants indicated as affected with nematodes. Twenty-three per cent of the total number developed galls, however, but none showed symptoms of the blight, altho the cultures were known to be virulent for the reason already stated.

TABLE 8.—*Influence of nematodes on the percentage of infection by* F. tracheiphilum.

Organism	Total number plants	No. with nematode galls	No. with *Fusarium* sp.
F. tracheiphilum from cowpea and nematodes..	10	10	2
F. tracheiphilum from cowpea without nematodes............................	10	0	0
F. tracheiphilum from soy bean and nematodes.	10	10	3
F. tracheiphilum from soy bean without nematodes............................	10	0	2
Nematodes only........................	10	10	0
None (control)..........................	20	0	0

A previous test had been made with soy beans, between September 26 and December 1, 1916, in an attempt to determine whether the presence of nematodes increases the number of infections. The nematodes were introduced into the soil of large buried pots in root galls from living soy beans free from infection by *Fusarium* spp. The results are presented in Table 8.

At Lincoln, on December 4, 1917, six stone jars of five gallons capacity were filled with nematode infested soil from a greenhouse bench in which badly nematode-infected tomatoes were growing. Four other jars were filled with nematode-free potting soil. Cultures of *Fusarium* growing on pieces of sweet clover *(Melilotus alba)* stems, from two 1 liter flasks, were incorporated into the soil of each jar. Three Clay cowpeas and three Mammoth Yellow soy beans were grown to maturity in each jar in a greenhouse kept at approximately 28° C. during the day and 22° C. at night. On February 16, when the plants had nearly matured seed pods, they were removed and examined. Table 9 shows the number of plants affected with nematodes and with *Fusarium*.

TABLE 9.—*Influence of nematodes on infection by* F. tracheiphilum.

Jar No.	Organisms	Total number of plants		Per cent affected with nematodes		Per cent affected with *Fusarium*	
		Soy bean	Cowpea	Soy bean	Cowpea	Soy bean	Cowpea
1	*Fusarium* and nematodes...	3	3	100	100	0	100
2	do	3	3	100	100	0	100
3	do	3	3	100	100	0	100
4	do	3	3	100	100	0	100
5	do	3	3	100	100	0	100
6	do	3	3	100	100	0	100
7	*Fusarium*.....	3	3	0	0	0	33⅓
8	do	3	3	0	0	0	66⅔
9	do	3	3	0	0	0	66⅔
10	do	3	3	0	0	0	33⅓

It is to be noted that all of the plants in the last test were infected with nematodes but that the cowpeas were more severely affected, the galls being somewhat larger and more numerous. The cowpeas generally showed symptoms of the fungus whether the eelworms were present or not. The soy beans remained free from the fungus in spite of nematode injuries. The galls on the soy beans did not reach a diameter of more than 1 to 2 mm. whereas in the fields where the blight developed they were commonly 8 to 10 mm. in diameter. The presence of the galls even tho small, and the absence of the fungus on the roots of plants in soil infected with both organisms in this and the two other tests mentioned, is evidence that the presence of nematodes under conditions which favor the development of the fungus on cowpeas does not supply the conditions that obtain in the field, which allow the development of this fungus on soy beans.

THE INFLUENCE OF SOIL TEMPERATURE

Recent reports of various investigators point out the relation of temperature to diseases caused by soil inhabiting fungi especially species of *Fusarium*. Jones (15) emphasizes the importance of this factor by summarizing the results of these investigations and correlating them with his own field observations. In main the results with *Fusarium* spp. show a minimum temperature for infection around 15° to 17° C. and a rather broad optimum of about 25° to 30° C. As far as the writer is aware, no data on maximum temperatures have been reported.

Before attempting to determine the influence of certain temperatures on soy bean blight, the organism was tested in culture at constant temperatures of 5°, 8°, 12°, 16°, 20°, 28°, and 33° C. After 10 days at 5° there was practically no growth, at 8° the colonies averaged 1 or 2 mm. in diameter, at 12° they were about 5-7 mm., at 16° about 18-20 mm., and with a rise in temperature to 28° a corresponding increase in size of colonies. At 33° the growth was somewhat less than at 28°. Cultures of the other wilt producing species of *Fusarium* reported by others correspond rather closely in their growth at these temperatures.

The optimum condition for infection in the case of soy bean blight may, however, be entirely different from that which is favorable to either parasite or host. Experiments are now in progress in which soy beans are growing in inoculated soil maintained at various constant temperatures. Nothing of significance, however, can at present be reported as to the relation of this factor, except that the amount of disease has not been increased at 14, 16, 17, or 24 degrees Centigrade.

THE INFLUENCE OF OTHER ORGANISMS

It is very possible that a larger number of infections and a more extensive development of the fungus within the host occur in the field than under the conditions of controlled inoculations because of the presence in the former of associated parasitic organisms and the injuries caused by them, or because of other injuries of the roots caused by insects, mechanical tools, or improper culture. This may oftentimes be of especial importance in connection with *Fusarium* spp., which are not as strict parasites as some and therefore enter more readily thru wounds. Two such organisms of the soil, namely, *Rhizoctonia* and *Sclerotium rolfsii*, were noticed affecting soy beans in fields where the *Fusarium* blight was abundant. The soy bean is more resistant to *Rhizoctonia* than most garden and field crops but lesions were noted on seedlings, young plants, and the small roots of older plants. *Sclerotium rolfsii*, however, is very destructive to soy beans whenever present and kills plants at all stages of their growth.

Soy beans are now growing in soil inoculated with *Fusarium tracheiphilum* and *Rhizoctonia*, and in another soil inoculated with *Fusarium tracheiphilum* and *Sclerotium rolfsii*, and it is believed by the writer that either these or other organisms will be found to play an important part in the development of this disease. It seems probable from preliminary tests that *Fusarium tracheiphilum* is unable to penetrate soy bean roots to the xylem elements unaided but that, once it gains entrance thereto, it develops rapidly in them in a somewhat saprophytic manner, obscures the primary injury, and finally affects the host as previously described.

FIELD EXPERIMENTS TO DETERMINE THE SUSCEPTIBILITY OF VARIETIES

Sixty per cent of the Mammoth Yellow soy beans in the field at Red Springs, N. C., were blighted in 1915. The main part of this field was planted to the same variety on May 23, 1916; but in another part reserved for the purpose, one 54-meter row each of Haberlandt, Mammoth Yellow, Pekin, Black Eyebrow, Medium Yellow Virginia, and Tar Heel Black soy beans and a row of Clay cowpeas were planted on June 8. On August 10 the main field and all of the varieties in the test, including the cowpeas, showed considerable blight or wilt, except the Black Eyebrow and the Virginia varieties of soy beans. On August 26 the latter of these varieties was apparently free from disease, but the plants had declined with age to such an extent that the exact determination was doubtful. The fact that these varieties were planted so late that hot, dry weather prevailed during a large part of their early growth explains why they were so rapidly forced to maturity. The Black Eyebrow variety remained free from disease thruout the season. An examination of plants in all parts of the field showed that the disease was abundantly and uniformly distributed.

Since the Black Eyebrow variety, which was planted late in 1916, i. e. June 8, remained free from the disease, it was planted again at intervals in 1917 in order to observe the effect of seasonal planting on the development of the disease. The first planting was made on April 26, another on May 31, and a third on June 21. The ground was hardly suitable for planting between these dates. Plants from the second and third planting were much dwarfed because of unfavorable weather, severe nematode infection, and a lack of cultivation, and for this reason no certain results as to the amount of infection in the different plantings were obtained. The most important determination possible from these observations is that some other variety more suitable to late planting should be used in future tests for this purpose. Since the Black Eyebrow variety remained free from the disease in 1916, it is important to note that a few diseased plants of this variety were found in the general variety test rows referred to below where the seed were planted earlier. However, the variety seems to show some evidence of resistance.

A larger number of varieties were tested in this field in 1917. Three rows each of the following varieties were planted on April 26: Brown, Black Eyebrow, Virginia, Mammoth Yellow, Early Dwarf Green, Wilson Black, Barchet, Jet, Austin, Arlington, Guelph, Chiquita, Auburn, Manchu, Tokio, Peking, Tar Heel Black, Haberlandt, and Medium Yellow. Every tenth row was planted to Clay cowpeas, and one row of the One Hundred Day Speckle velvet bean *(Mucuna utilis)* was planted around two sides of the field. By July 20 the disease was abundant in all of the varieties of soy beans except the Black Eyebrow discussed above. The cowpeas were so badly diseased that all died before blooming. The velvet beans were entirely free from nematodes or *Fusarium*.

The Brown variety, altho as badly infected by the nematode and *Fusarium* as any of the other varieties, deserves special mention because of its tolerance to these parasites. In spite of the presence of a large number of nematode galls, varying in diameter from 1 to 15 mm., and the presence of *Fusarium* thruout the xylem area of the roots and for some distance up the stem, there was absolutely no wilting, no chlorosis, nor any external evidence of any infection whatsoever. The plants were from four and one-half to five and one-half feet in height, they stood six inches to a foot above any other variety and more than that above the Mammoth Yellow; they remained green and healthy in appearance thruout the season and produced a good crop of beans which were about mature on September 8, when the field was pastured to hogs. Perhaps the size of these plants was somewhat reduced by the action of the two organisms. Unfortunately there were no check plants with which these could be compared since the entire field was infected. Affected plants of this variety, however, yielded as well as the average healthy Mammoth Yellow plants in nearby fields.

The Brown soy bean is identical with the Mammoth Yellow in habit-type and is very similar to it in other respects. The color of the seed is the only apparent difference. This variety has been grown but very little in North Carolina, but tests have shown that it is as desirable in every respect, if not more so, than the present favorite variety, the Mammoth Yellow, which suffers greatly from *Fusarium* blight. Mr. Walter White at Edenton, N. C., says that he prefers the Brown to the Mammoth Yellow because it produces more forage and more seed and can be grown wherever conditions are favorable to the Mammoth Yellow.

The Haberlandt variety, which is also suitable to conditions in North Carolina, develops well in spite of rather severe *Fusarium* and nematode infection.

The Brown and Haberlandt varieties are preferable to the Black Eyebrow for planting in infected soil, especially if nematodes are present, even tho the latter should remain free from *Fusarium* infection.

SUMMARY

I. A disease of the soy bean has been studied during the past three years. The first report of this disease appeared in a publication by the writer in 1917.

II. The disease is characterized by a chlorosis and shedding of the leaves or leaflets, followed by the death of the plants, and is herein called "blight."

III. Soy bean blight has been observed in several localities within North Carolina on soils infested with cowpea wilt. What is probably the same disease has been recently observed by others in Alabama and possibly in Wisconsin.

IV. A species of *Fusarium* belonging to the section *Elegans* is the causal organism.

V. Cultural and morphological studies which are regarded as of primary importance in distinguishing species of *Fusarium* show that the strain of *Fusarium* on soy bean is identical with the organism producing the wilt of cowpeas.

VI. Reciprocal inoculation experiments with the strains from soy beans and cowpeas show that cross-inoculations can be made. These experiments were conducted in the greenhouse and under field conditions. Pure cultures of the two strains were used in certain of the experiments and inoculum from the natural host in others.

VII. Blight of soy beans is therefore due to *Fusarium tracheiphilum.*

VIII. Physical structure and acidity of the soil under natural conditions are not the limiting factors in infection, but acidity under certain conditions may have some influence.

IX. Infection occurs thru the roots, but nematodes appear not to increase the percentage of blight materially. Other organisms such as *Rhizoctonia* and *Sclerotium rolfsii* and other root injuries are believed to materially increase the percentage of diseased plants in the field.

X. The Black Eyebrow variety of soy beans shows some evidence of resistance. The Brown variety, while not resistant, is tolerant and seems to develop remarkably in spite of numerous fungous filaments and nematodes within the roots. Fifteen other varieties tested are severely affected. Velvet beans are not subject to infection.

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