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Studies of a Seedling Blight of Soybeans and the Etiology of the Causal Fungus, *Diaporthe phaseolorum* var. *caulivora*[1]

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Diaporthe phaseolorum var. *caulivora* is a fungus that can infect soybeans (*Glycine max*) and cause development of stem cankers that usually result in girdling of stems and death of plants. Stem canker disease of soybean was first described by Welch and Gilman (11) when they differentiated the stem canker fungus from *D. phaseolorum* var. *sojae*, which causes pod and stem blight of soybean. Welch and Gilman first identified the causal organism of stem canker as *D. phaseolorum* var. *batatatis*, a fungus described by Harter and Field (6) as being pathogenic on sweet potato. Athow and Caldwell (1) revised the variety name to the one in current use because of differences in morphology and pathogenicity between cultures of *D. phaseolorum* var. *batatatis*, known to be pathogenic on sweet potato, and the stem canker fungus.

Wolf and Lehman (12) first reported pod and stem blight as a disease of soybean in 1920. The disease was observed to cause damage to soybeans sporadically until the 2 varieties of the fungus were separated by Welch and Gilman in 1948. After this date the stem canker disease only has been reported to cause killing of plants.

The stem canker fungus has been reported to be seed transmitted by Frosheiser (4) and Hildebrand (7), although the percentage of infected seed obtained was quite low. Frosheiser found only 5.1 per cent infected seeds when he attempted to isolate the fungus from seeds from pods formed on or within 2 inches of the canker. He was unable to isolate the fungus from the remaining seed taken from infected plants. Hildebrand (7) found no increase in the incidence of stem canker when he compared plants grown from seed from infected and healthy plants. However, the possibility remains that a small percentage of infected seed may give rise to diseased seedlings.

As early as 1923, Lehman (8) observed that the pod and stem blight fungus growing on seed coats was infecting soybean seedlings.

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Considering that we now know there are 2 varieties of the same fungus infecting soybeans, it remains uncertain which fungus Lehman was observing. Thus, there is a possibility that either fungus, or both, may cause a soybean seedling rot. Gerdeman has reported the pod and stem blight fungus associated with root and basal stem rot of soybeans (5). Because the stem canker fungus is generally considered to be more pathogenic than the pod and stem blight fungus (1, 2, 3), the following study was conducted to determine if the stem canker fungus was causing a seedling blight of soybeans and, if so, to obtain information on the etiology of the fungus.

Materials and Methods

The effect of *D. phaseolorum* var. *caulivora* on soybean seedlings in the greenhouse was determined in steamed and nonsteamed soil. The soil used was a dark silt-loam. The steamed portion was autoclaved at 20 pounds pressure for 4 hours. Plants were grown in 4-inch clay pots. All seeds were planted at a depth of 1 inch and inoculum was placed in various positions in relation to the seeds. Inoculum consisted of the fungus growing on boiled soybeans. Six pieces of inoculum consisting of individual soybeans were placed uniformly in each pot. The soil in which control plants were grown contained no inoculum. Ten seeds were sown in each pot and 5 pots of soil were included in each treatment. The variety Hawkeye was used for all tests.

Inoculum for field tests consisted of stems of soybean plants bearing perithecia of the pathogen. These stems were obtained from infected plants the previous autumn. Furrows were opened in the rows and the stems were placed in the furrows parallel to the rows. Seeds were placed adjacent to the stems at 1 inch intervals and both inoculum and seeds were covered with 1 inch of soil. Other rows contained sections of healthy stems or no stems. Rows were 10 feet long and there were 4 rows of each treatment included in the test.

Potato-dextrose agar was the only nutrient agar medium used in the studies dealing with the effect of temperature and pH on the growth of the fungus. This medium was adjusted to pH 7 unless otherwise stipulated. For the temperature and rate of growth studies on nutrient agar, the medium was slanted in 8 x 1 inch test tubes. The fungus was transferred to the medium near the end of the tube on an agar disc 4 mm. in diameter. The lateral growth of the fungus was measured from the edge of the disc to the tip of the most advanced hyphae. For the studies of the effect of substrate pH on fungus growth and of fungus growth on soil, Petri dishes were used. An agar disc on which the fungus was growing was placed on the medium in the center of the dish and the diameter of the fungus mat recorded.

Organic matter content of soils was determined by heating soil samples to red heat for 15 minutes and determining loss in weight of the samples. Organic matter added to soils was in the form of a coarse powder prepared by grinding dried soybean leaves in a Wiley mill.

Soil used as a medium for fungus growth was Webster silty-clay loam obtained from the upper 2 inches of a plowed field.

In the experiment dealing with the overwintering of the fungus, stems bearing perithecial initials were placed in 4-quart perforated polyethylene plastic bags with sufficient soybean straw to surround the stems, and the bags were buried in field soil to a depth of 3 to 7 inches. In another experiment, round, metal cans, 7 inches tall and 4½ inches in diameter were filled with field soil. Ten cans of soil were included in the experiment and each was plugged at the upper end with a piece of cotton batting. Soil moisture was adjusted to 15 per cent before the soil was added to the cans. All cans were then weighed. Five cans of soil were autoclaved twice for 2 hours, reweighed, and sufficient sterile water added to restore soil moisture lost in autoclaving. Four sections of soybean stems bearing perithecial initials were inserted in each can of soil. The openings of the cans were sealed with 5-inch squares of heavy aluminum foil, and all cans of soil were frozen and stored at —18°C.

Development of perithecial initials in the dark was studied by use of a light-proof, black, plastic box. The interior of the box was lined with moist, heavy paper blotters. The stems were placed on inverted glass dishes in the bottom of the box. Inverted glass dishes were placed on top of the soybean stems and the dishes were then covered with moist blotting paper. The box lid was taped in place and the box was stored in a temperature chamber at 25°C.

A single ascospore isolate of the fungus was used throughout all cultural studies. In studies of the effect of light on fungus development, the source of light was a single, 75 watt, incandescent light bulb 5 feet above the cultures. The fungus was grown in 500 ml. flasks containing 200 ml. of potato-dextrose broth at a temperature of 20°C.

Results

Field Observations

Hawkeye soybean seedlings 3 weeks old were examined in a field where stem canker diseased plants had grown for the preceding 2 years. One hundred seedlings were examined in each of 10 locations in the field. Sixty-one diseased seedlings were found among the 1,000 examined. An attempt was made to isolate causal organisms from these diseased seedlings. Organisms isolated and percentage of

plants infected were: *D. phaseolorum* var. *caulivora*, 0.8 per cent; *Fusarium sp.*, 2.1 per cent; *Pythium sp.*, 1.4 per cent; miscellaneous bacteria, 0.8 per cent; *D. Phaseolorum* var. *sojae*, 0.2 per cent; *Rhizoctonia solani*, 0.1 per cent; and undetermined cause of disease, 0.7 per cent. Of the diseased plants examined, only those infected by *Pythium sp.* could be identified from the others. Pythium-infected plants tended to have a watery appearance. The majority of the plants infected with the stem canker fungus had reddish-brown lesions on 1 or both cotyledons (Fig. 1). The fungus was also isolated from roots and occasionally from stems of seedlings. The morphology of the fungus was the same as that described for *D. phaseolorum* var. *batatatis* by Wehmeyer (10) and Welch and Gilman (11), and for *D. phaseolorum* var. *caulivora* by Athow and Caldwell (1). The field had been sown with certified seed and an

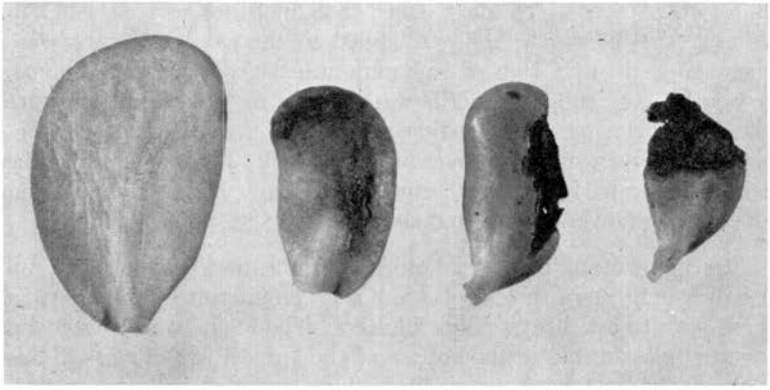

Figure 1. The three soybean cotyledons at right are infected with *Diaporthe phaseolorum* var. *caulivora*, while the cotyledon at left is not infected.

attempt to isolate the fungus from 1,000 seeds of the same seed lot failed.

The stem canker phase of the disease usually does not occur until the later part of July. The presence of the disease on seedlings in the spring may be the initial phase of the disease that is followed later by canker formation. The presence of the disease so early may also be important in building up fungus inoculum.

Greenhouse Experiments

An experiment was conducted to determine how placement of inoculum in soil affected disease development, and to ascertain whether the diseased condition as observed in the field could be duplicated under greenhouse conditions. Inoculum was placed one-half inch above the seeds, directly above the seeds, directly below the seeds and one-half inch below the seeds. Plants were grown for

6 weeks in a greenhouse in which the daytime temperature was 27°C. and the nighttime temperature 20°C.

The greatest reduction in germination occurred when inoculum was placed one-half inch above the seeds in steamed soil (Table 1). There was more reduction in germination in steamed soil than in nonsteamed soil in all treatments except the control. The fungus grew much more rapidly through the steamed soil than through the nonsteamed soil presumably because of the absence of other microorganisms. Thus, a higher percentage of seedlings in the steamed soil were killed before emergence.

Table 1

Average Response of 5-Week-Old Hawkeye Soybean Plants Exposed to *D. phaseolorum* var. *caulivora* in Steamed and Non-Steamed Soil Under Greenhouse Conditions

Location of inoculum	Average[1] percentage germination	Average[1] percentage infection	Average[1] plant height (mm)	Average[1] disease rating of roots[2]
½ inch above seeds				
steamed soil	30	100	129	4.4
nonsteamed soil	56	82	150	3.6
directly above seeds				
steamed soil	48	96	130	4.0
nonsteamed soil	56	90	141	3.2
directly below seeds				
steamed-soil	63	96	204	3.3
nonsteamed soil	68	30	199	2.8
½ inch below seeds				
steamed soil	84	96	204	2.5
nonsteamed soil	88	82	244	2.0
control (noninoculated)				
steamed soil	96	0	252	1.0
nonsteamed soil	94	0	250	1.0

[1]Average of 5 pots.
[2]Numerical disease rating ranged from 1 (healthy) to 5 (severely rotted).

A higher percentage of plants that emerged was infected in the steamed than in the nonsteamed soil. Percentages of infection were high in all infested, nonsteamed soils with the exception of the treatment in which the inoculum was directly below the seeds. The reason for obtaining 96 per cent infection in steamed soil in comparison with 30 per cent in nonsteamed soil is unknown. It was noted, however, that there was less infection of cotyledons and stems when inoculum was placed under the seeds than when placed above them. It was also noted that older portions of roots were more resistant than the tissues in the vicinity of root tips. Since the fungus developed slowly in nonsteamed soil, the root tissues first reached by the fungus may have developed mechanical resistance to infection prior to the time the fungus reached the roots. This situation was evidently not true when inoculum was placed one-half inch below seeds in nonsteamed soil, because the average percentage infection was 82 per cent in this case.

In all treatments involving infested soil, except that in which inoculum was placed directly under seeds, plants grown in nonsteamed soil were taller than those grown in steamed soil. In general, average plant height was greater when inoculum was placed below the seeds than when it was placed above them. There was considerable variability in height of plants grown in infested soil (Fig. 2).

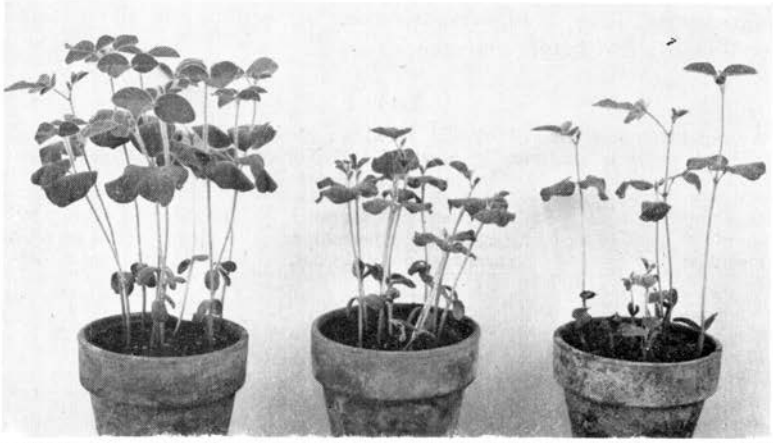

Figure 2. Comparison of vigor of healthy soybean seedlings (pot at left) and seedlings infected by the stem canker fungus.

In all treatments except the control, plants grown in steamed soil were more severely diseased than those grown in nonsteamed soil. Infection of cotyledons and stems was much more severe when inoculum was placed above seeds than below. This also resulted in more severe stunting of plants and is an indication that stems and cotyledons of soybean seedlings are more susceptible to rotting by *D. phaseolorum* var. *caulivora* than roots.

Plants were grown in sterile soil infested with stem canker inoculum placed above surface sterilized seeds. Soil was contained in 4-liter flasks with mouths stoppered with cotton plugs. This allowed the plants to emerge and grow in a very humid atmosphere varying from 90 to 100 per cent relative humidity. Many cotyledons were partly or completely covered with dense, white mycelium that grew into stems. Shortly after stems were infected, the plants were killed. Damage to cotyledons and stems was much more severe when infected plants were grown in a very humid atmosphere than in the less humid greenhouse atmosphere.

Field Experiment

To determine whether seedlings could be infected by growing them adjacent to soybean stems infested with the stem canker fungus, the

following experiment was conducted. Soybean seed was sown in contact with buried stems from infected plants and with buried stems from healthy plants. Seed was sown in control rows that contained no stems. Average percentage germination, height and percentage infection was recorded 4 weeks after the seed was sown (Table 2).

Table 2

Average Percentage Germination, Height and Percentage Infection of Hawkeye Soybean Plants After Seeds Were Sown in a Field in Such a Way That Seed Contacted Soybean Stems Bearing Perithecia of *D. phaseolorum* var. *caulivora*, and Healthy Stems.

	Average[1] percentage germination	Average[1] plant height (mm)	Average[1] percentage infection
stems with perithecia	82	74	61
healthy stems	90	84	0
control	92	86	0

[1]Average of 4 rows.

Average percentage germination and average plant height were reduced slightly in rows in which seed was sown adjacent to stems with perithecia. Average percentage infection was 61 per cent in these rows. No infected plants were found in any of the remaining rows. Roots of infected plants had been rotted extensively in many cases and in a few cases the primary root had been rotted completely (Fig. 3).

Results of greenhouse and field experiments demonstrate that *D. phaseolorum* var. *caulivora* can cause a seed and seedling rot of

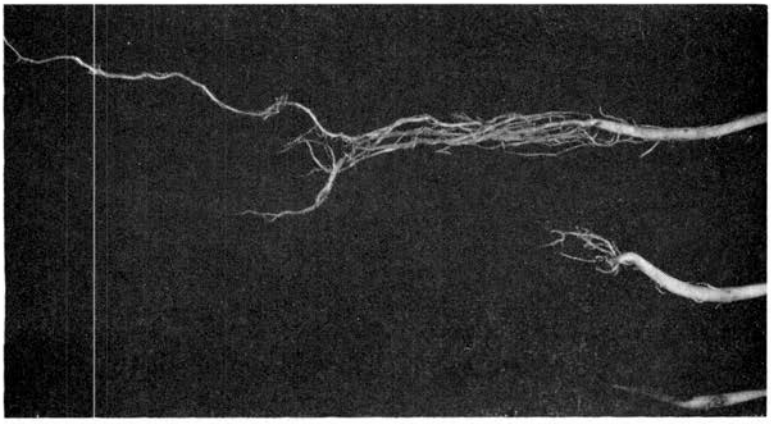

Figure 3. Roots of a healthy soybean seedling (top) and roots infected with *D. phaseolorum* var. *caulivora* (center and bottom).

soybeans. Although this phase of the disease was observed to be relatively uncommon, it could be important when growers attempt to grow soybeans a second year in fields that contained a high percentage of infected plants, since stand and plant vigor are directly affected.

Cultural Studies of the Fungus

Growth of *D. phaseolorum* var. *caulivora* was studied at temperatures ranging from 5° to 30° C. (Fig. 4.). Lateral growth of the fungus in millimeters was recorded at intervals for each temperature studied. It was rather surprising that the fungus made any growth at 5° C. At 30° C. growth proceeded at a fairly good rate for 5 days and then decreased sharply. The mycelium was white at all temperatures except at 30° C., when the older mycelium became light brown. The decrease in growth rate after 5 days at 30° C. may be explained by the accumulation of staling products in the medium.

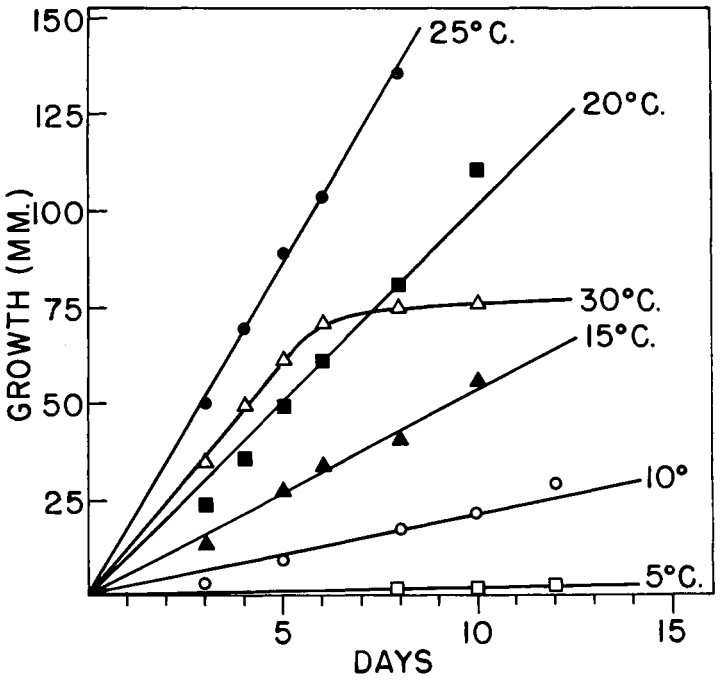

Figure 4. Lateral growth, in millimeters, of *D. phaseolorum* var. *caulivora* on potato-dextrose agar at temperatures ranging from 5 to 30° C.

Rate of growth of the fungus increased slowly from 5 to 15° C. and rapidly from 15 to 25° C. (Fig. 5). Optimum temperature for

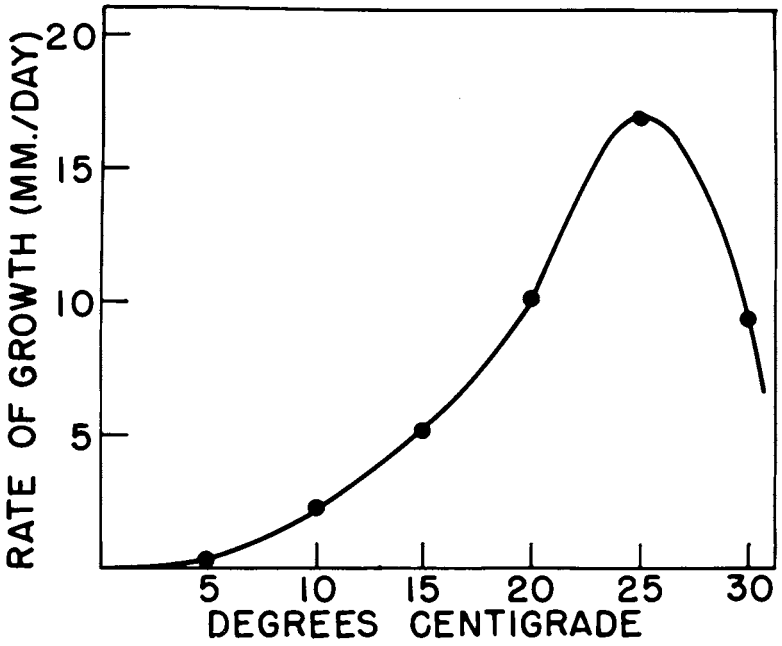

Figure 5. Rate of growth, in millimeters per day, of *D. phaseolorum* var. *caulivora* on potato-dextrose agar at temperatures ranging from 5 to 30° C.

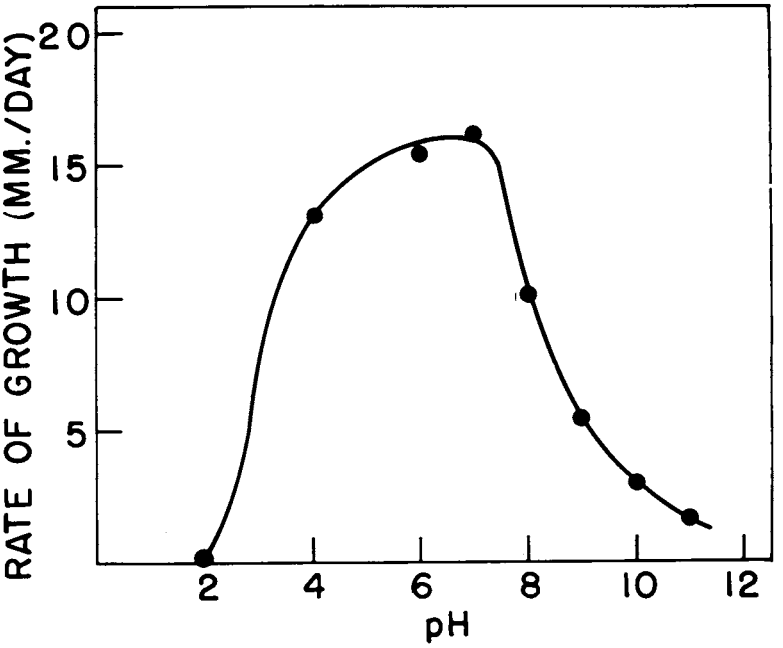

Figure 6. Rate of growth, in millimeters per day, of *D. phaseolorum* var. *caulivora* on potato-dextrose agar at pH values ranging from 2 through 11.

lateral growth of the fungus was 25° C. Growth rate dropped sharply between 25 and 30° C.

The fungus was grown on a medium that had been adjusted to pH values ranging from 2 through 11. Rate of growth of the fungus increased sharply from pH 2 to pH 4 (Fig. 6). Rate of growth was at a maximum between pH 6 and 7, and decreased rapidly between pH 7 and 8.

Sterile and nonsterile field soil provided enough nutrients to maintain the fungus for several months provided it was kept sufficiently moist. The fungus formed perithecia on soil and produced viable ascospores in 30 to 35 days at room temperature. Mycelial development was much less dense on soil than on potato-dextrose agar medium; however, it became quite dense in areas of perithecial formation. Since the fungus grows well on soil and has been found to infect soybean seedlings, there is little reason to doubt that the fungus is present in soils in which infected soybean plants have been grown.

Observation of growth of the fungus on soil indicated that rate of growth was influenced by soil moisture. The fungus grew very slowly, or not at all, in dry soil, and it also grew slowly in saturated soil. Oven dried, sterile field soil containing from 5 to 30 per cent water was used as a medium, and rate of growth of the fungus was recorded (Fig. 7). There was a very rapid increase in rate of growth between 5 and 15 per cent water content. The rate of growth increased

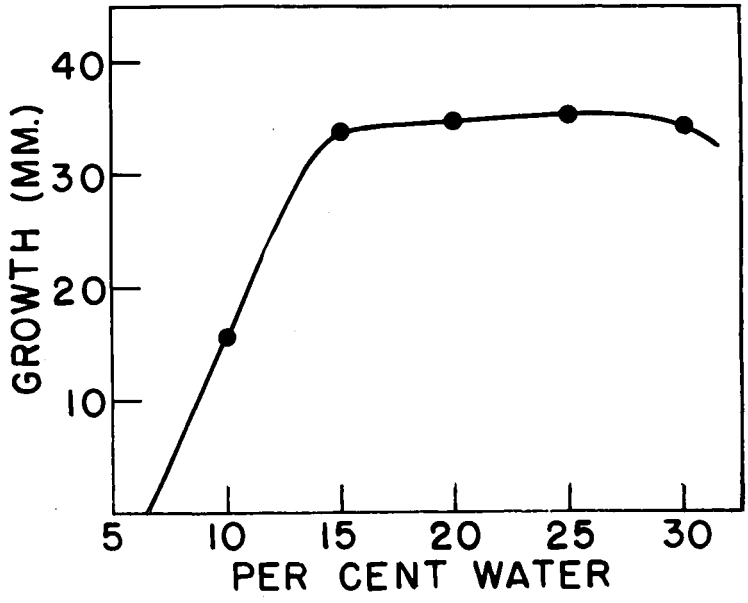

Figure 7. Growth, in millimeters, of *D. phaseolorum* var. *caulivora* in soil when water content ranged from 5 to 30 per cent.

slightly between 15 and 25 per cent water content and decreased slightly between 25 and 30. Optimum water content for lateral growth of the fungus was 25 per cent.

The fungus was grown on sterile soil containing 20 per cent water, at room temperature for one week. The soil was allowed to dry at room temperature for an additional week, after which, an attempt was made to reisolate the fungus. This experiment was repeated twice and in each case the fungus could not be isolated from the soil.

The organic matter content of field soils, in which the fungus was observed to grow, varied from 0.5 to 1.8 per cent. All soils were obtained in the spring from the upper 2 inches of newly plowed and disced fields. Additional organic matter in the form of ground, dried leaves was added in various quantities to soil that had an organic matter content of 0.5 per cent. Soil moisture was 25 per cent. The fungus was grown on the soil at 20° C. and total lateral growth determined after 8 days. The percentage of organic matter added to soil and the average lateral growth of the fungus in 5 plates at each percentage were as follows: 0.0 per cent, 25 mm.; 0.5 per cent, 27 mm.; 1.0 per cent, 28 mm.; and 2.0 per cent, 42 mm. The fungus made the best growth on the soil with the highest organic matter content. Rate of growth on this soil was approximately one-half that recorded on potato-dextrose agar at the same temperature.

D. phaseolorum var. *caulivora* was cultured at temperatures rang-

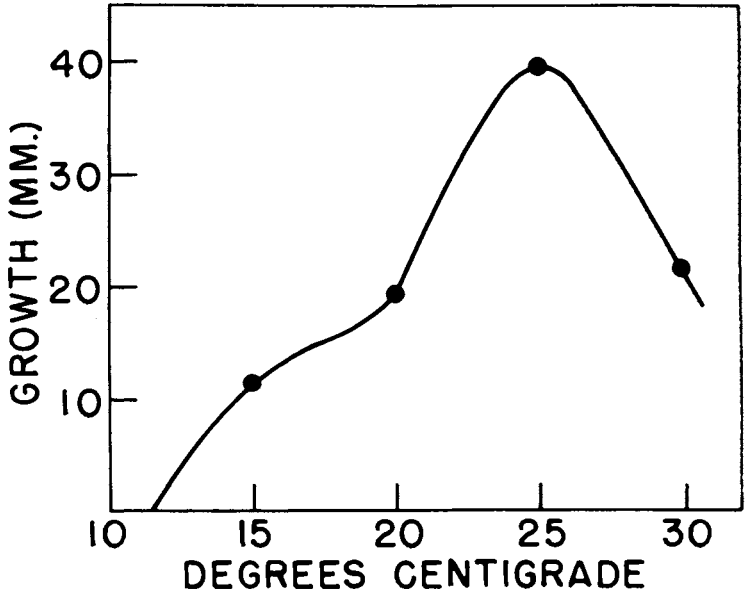

Figure 8. Growth, in millimeters, of *D. phaseolorum* var. *caulivora* in soil containing 1.5 per cent organic matter and 25 per cent water, at temperatures ranging from 10 to 30° C.

ing from 5 through 30° C. on soil having a total organic matter content of 1.5 per cent (one per cent added) and a water content of 25 per cent. Lateral growth of the fungus was recorded after 8 days (Fig. 8.). No growth of the fungus was observed at temperatures of 10° C. or lower. The rate of growth increased rapidly between 20 and 25° C. and decreased rapidly between 25 and 30° C. Rate of growth on this soil was about one-third that recorded for potato-dextrose agar at 25° C.

Overwintering of the Fungus

Timnick et al. (9) have shown that perithecial development is greatly inhibited when the fungus grows in total darkness. They reported an average of only 3 perithecia per plate when the fungus was grown in total darkness, as opposed to 1,500 when grown in alternate light and darkness. Because diseased stems are plowed under, thus being placed in total darkness, the possibility exists that perithecial development would not proceed. To investigate this point, diseased stems taken from mature, field grown plants were placed in a moist chamber in total darkness. Numerous perithecial initials occurred on stems before they were placed in the moist chamber. The stems were examined 30 days after being placed in the moist chamber, and had fully formed perithecia that produced viable ascospores in profusion.

To determine if the fungus isolated in Iowa behaved similarly to that described by Timnick, a number of experiments on the effect of light on fungus growth were conducted. Average lateral growth on 5 plates of potato-dextrose agar after 5 days at 20° C. was 38 mm. when the cultures received 8 hours of light per day, and 16 mm. when they received continuous light. The mycelium of cultures grown in continuous light was sparse and closely appressed to the substrate, as opposed to that grown in alternate light and darkness, which was dense and had numerous aerial hyphae.

Average dry weight of 5 mycelial mats grown in potato-dextrose broth in continuous light for 2 weeks was 0.16 g., that in 8 hours of light per day was 2.41 g., and that in continuous darkness was 1.95 g. The cultures receiving 8 hours of light per day had formed numerous perithecial initials when the experiment was terminated, whereas the cultures in continuous light or darkness had formed none. It was thus established that alternate light and darkness are essential for rapid formation of numerous perithecial initials, and that once the initials are formed, perithecia develop and produce ascospores on soybean stems in continuous darkness.

Soybean stems with numerous perithecial initials were buried in 3 perforated plastic bags in early November. The contents of one bag was examined March 1, at which time none of the perithecial initials had begun to develop beaks. The stems in the second bag were ex-

amined April 1, when approximately 60 per cent of the clusters of perithecial initials had begun to develop beaks. The stems in the third bag were examined May 1, and by this time slightly over 80 per cent of the perithecia had formed beaks that were overgrown with a heavy mat of mycelium. Viable ascospores were recovered from these perithecia.

Stems with perithecial initials were thoroughly washed and placed in cans of sterile and nonsterile soil. All cans were stored at —18° C. One can of sterile and one of nonsterile soil were removed from cold storage after one month and the remaining 8 cans after 14 months. Cans were then stored at room temperature and soil moisture maintained at approximately 15 per cent. Perithecia and viable ascospores developed on all stems in both sterile and nonsterile soil and mycelium grew throughout the soil.

Although it had been established that the fungus could overwinter on soybean straw either on the surface or buried, it remained to be determined if the fungus could overwinter as mycelium in soil. The fungus was grown on tubed slants of potato-dextrose agar and tubes of sterile field soil containing 20 per cent water for one week before storage at —15° C. for 3 months. Mycelium transferred from both the agar and the field soil, after removal from cold storage, gave rise to vigorous fungus cultures. Critical examination of the cultures prior to freezing showed no spores or observable development of perithecial initials.

Discussion

D. phaseolorum var. *caulivora,* as well as *D. phaseolorum* var. *sojae,* has been observed to parasitize soybean seedlings. Although observations of the seedling blight caused by either variety of the fungus have not been common, the condition may be a fairly common one but escapes detection because infected seedlings are scattered and because germinating seeds, if seriously infected, are killed before emergence. Similarity of symptoms of the disease and those caused by other seedling diseases make detection difficult. The field sampled for presence of the disease yielded only 0.8 per cent infected plants. The important point is not the amount of infection that occurred but that it did occur. The seedling blight phase of the disease is probably unimportant from the standpoint of decrease in stand and seedling vigor in most soybean fields because adjacent, healthy plants compensate for the occasional seedling that fails to emerge or grow rapidly. However, if an association could be established between the seedling blight phase of the disease and the stem canker phase, the importance of the former would be greatly increased.

The establishment of the fact that plowed-under soybean stems containing perithecial initials can develop and produce ascospores is

important not only as a means of overwintering for the fungus but also in its local spread in the soil. Rain water draining through plowed field soil in the early spring would probably distribute the ascospores in the soil below the perithecia. Since fields are prepared for planting after May 1 in Iowa, the fungus would likely be further distributed in discing and harrowing operations. Since the fungus can withstand temperatures as low as —15° C., it may be possible for it to overwinter in the mycelial stage.

Once the fungus has been established in soil, it can form perithecia and ascospores. Since alternate light and darkness are required for production of perithecial initials, perithecia and ascospores may be produced at the soil surface. The fungus develops best at 25° C. on moist, neutral or slightly acid soils high in organic matter content. Alkaline or dry soils are decidedly unfavorable for fungus growth.

Since it has been established that the fungus can parasitize roots of soybean seedlings, it seems logical that the fungus might also infect young roots of older plants as long as soil conditions remain favorable for fungus growth. For these reasons, investigations of the possible relation between root infection and later development of stem cankers should not be abandoned.

Summary

Diaporthe phaseolorum var. *caulivora* can cause a seedling rot of soybeans. Infection of cotyledons, stems, and roots was more severe when inoculum was placed in soil above seeds than below. Damage to cotyledons and stems was severe when infected plants were grown in a very humid atmosphere. Average percentage infection in rows in which seed was sown adjacent to stems with perithecial initials was 61 per cent.

Optimum temperature for lateral growth of the fungus was 25° C. and rate of growth was at a maximum between pH 6 and 7. The fungus formed perithecia on soil and produced viable ascospores in 30 to 35 days. Optimum water content of soil for lateral growth of the fungus was 25 per cent. Best fungus growth on soil was obtained on soils with high organic matter content.

Alternate light and darkness were required for production of perithecial initials, but mature perithecia and viable ascospores developed from perithecial initials in total darkness. The fungus may overwinter on infected soybean stems and as mycelium in soil.

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