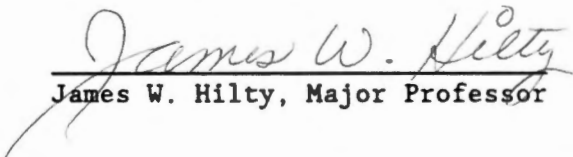
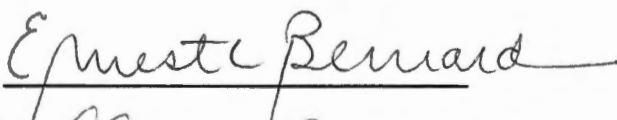
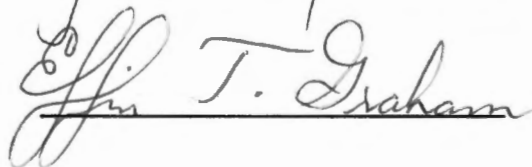


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THE EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT OF
DIAPORTE PHASEOLORUM VAR. CAULIVORA IN CULTURE

A Thesis

Presented for the

Master of Science

Degree

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Kimberly F. Goode

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DEDICATION

This thesis is dedicated to my parents, Faye and Jim Goode, in appreciation of the many years of continued support and encouragement which they have given.

ACKNOWLEDGMENT

The author would like to thank her major professor, Dr. Jim W. Hilty for his continued support throughout this long process and also thanks is extended to Dr. Ernest C. Bernard and Dr. Effin T. Graham for serving as committee members.

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ABSTRACT

The ascigerous state of Diaporthe phaseolorum var. caulivora (DPC), the causal organism of soybean stem canker, was studied under laboratory conditions to determine the effects of temperature and relative humidity on the development of perithecia and subsequent ascospore discharge. Discharge of the ascospores under controlled conditions of temperature and humidity was monitored in order to clarify aspects of the epidemiology and distribution of the soybean stem canker pathogen. Development of the perithecia, on sterile soybean stems, embedded in water agar culture, occurred between 20-26 C with an optimum at 22 C. Growth of DPC in culture increased with increasing temperatures from 20 to 26 C. Discharge of ascospores from three-week old perithecial cultures was stimulated by relative humidities above 55% at 22-23 C. Humidities between 70-100% favored a greater number of ascospores to be released. Histological studies were made in an attempt to learn more about the growth of the pathogen within the host. Ascospores were germinated on excised soybean stems in moist chambers and samples were taken at two day intervals to observe the stages of perithecial development. Light microscopy and histological studies revealed hyphal tips penetrating the epidermis and ramifying throughout the parenchyma cells of the stem tissue. The first recognizable structures to be formed were stromatal initials.

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I. INTRODUCTION AND LITERATURE REVIEW

Soybean stem canker, caused by Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell (DPC), is an economically important disease in the southeastern United States, responsible for large reductions in yield of soybeans (4,30,36,38). The term "stem canker" was first used for this disease by Crall (5) in 1950. The history of stem canker can be traced in the literature by following the disease complex known as pod and stem blight.

Stem canker disease of soybean is one of a group of disorders caused by members of the Diaporthe-phaseolorum complex. Diaporthe phaseolorum var. caulivora has become the subject of intensive research in the past ten years, primarily because of its ability to kill the soybean plant well before harvest.

Harter (12), in 1917, determined that D. phaseolorum (Cke. and Ell.) Sacc. was the cause of pod blight of lima bean. Lehman (32), in 1923, described a disease which he called pod and stem blight of soybean and gave the name D. sojae to the causal organism. Harter and Field (13), 1912, described the organism causing a sweet potato dry rot and named it D. batatatis. Wehmeyer (50), in his monograph on the genus Diaporthe, stated that D. sojae and D. batatatis did not differ from D. phaseolorum enough to be called separate species. He assigned the names D. phaseolorum (Cke. and Ell.) Sacc. var. sojae (Lehman) Wehm. and D. phaseolorum (Cke. and Ell.) Sacc. var. batatatis (Harter and Field) Wehm., respectively, to these two organisms. D. sojae Lehman was

described later (32), but this was renamed D. phaseolorum (Cke. and Ell.) Sacc. var. sojae (Lehman) Wehm. (10).

The first indication in the literature that stem lesions, which cause wilting and death of the plants, and typical pod-and-stem-blight symptoms were caused by separate organisms, is found in the report by Welch (51). He found that isolates from the stem lesions produced perithecia on potato-dextrose agar, while isolates from plant parts having typical pod-and-stem-blight symptoms produced pycnidia and conidia of the Phomopsis type on the same medium.

However, it remained for Athow and Caldwell (1) to report the occurrence of two closely related, but morphologically and pathologically different members of the genus Diaporthe on soybeans, and to state that the diseases caused by them are quite distinct from one another. The stem canker fungus was named Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell. The name of the pod and stem blight fungus remained unchanged. Until quite recently, the validity of this separation into two distinct varieties of Diaporthe remained unchallenged; however, Threinen et al., (47) stated that the separation of the two varieties of Diaporthe by morphological differences in culture and differences in pathogenicity on soybeans was invalid. They showed that mutants of the variety caulivora can produce symptoms on soybean plants indistinguishable from symptoms produced by the pod and stem blight fungus, and that upon crossing mutants of the variety caulivora with the variety sojae, the perithecia produced had the morphological characteristics of the variety sojae.

During the past 10 years, significant stem canker damage to soybeans has been reported in the southern United States (4,38). In all these states, plantings of some cultivars were almost destroyed, whereas other cultivars either were unaffected or were damaged at intermediate levels (26). These reports suggest that major differences in severity are epidemiologically based and controlled by factors poorly identified. The recent report by Keeling (27) of six races of DPC indicates its potential for further adaptation and spread into previously unaffected areas. Keeling suggested that there are six physiological races that could be differentiated by the cultivars Kingwa, Tracy-M, Arksoy, Centennial, S-100 and J77-399, with northern isolates classified as race 4, 5 or 6 and southern isolates as race 1, 2 or 3. Keeling also observed that some northern cultivars were susceptible to southern isolates.

There appear to be insufficient grounds for maintenance of var. caulivora as a separate taxonomic entity. The strains associated with stem canker can, however, be consistently distinguished from others of D. phaseolorum. Kulick (31) has advocated that the strains causing soybean stem canker be referred to as forma specialis caulivora to indicate association with a distinct disease condition. This recognizes that the distinction mainly involves physiological reaction, although this may be accompanied by slight morphological differences, especially in cultural characteristics in vitro.

Backman et al. (3) and Morgan-Jones (37) have observed cultural and morphological differences between northern and southern biotypes. Overall colony surface appearance, stromata size, perithecial beak morphology, ascospore shape, conidiophore branching, production of alpha

or beta conidia predominantly and radial growth rate at 30 C are all criteria where stable discontinuities exist among isolates from different geographic origins. Their results indicate that observed differences, together with differing physiological capacity to induce stem canker, might justify separation of northern and southern races into separate forma specialis of D. phaseolorum. Current research indicates that the aggressive strains of DPC found in the southern United States are different from their northern counterparts. McBee and Biddle (36) agreed with Backman et al. (3) but also observed an additional difference in the presence of chlamydospores in southern D. phaseolorum cultures. This is the first report of their occurrence in D. phaseolorum.

Hobbs and Phillips (19) observed subtle differences between symptoms on field-grown plants in the Southeast and symptoms on plants in the Midwest. They also reported differences in growth rate and colony appearance between DPC and the undetermined Diaporthe sp. causing stem canker in the Southeast. They suggested that the latter disease be referred to as "southern stem canker" and that isolates of the pathogen be called "southern isolates of Diaporthe phaseolorum causing stem canker."

Symptomatology

Soybean plants are susceptible to infection, with earliest symptoms developing on seedlings. The disease is characterized by brown-black shallow cankers usually beginning near a lower leaf node. However, symptoms are rarely seen when plants are less than 70 days old (16). As the disease develops, these lesions darken, elongate and become a sunken

canker that often girdles the stem. When girdling occurs it prevents further development of pods and seeds and causes leaf symptoms. Leaf symptoms appear as interveinal chlorosis and necrosis. Eventually the whole plant dies, with the dried leaves being retained on the stems.

Overwintering

The causal organism of stem canker, DPC overwinters as perithecia, which occur in groups of 2-12 per stroma, with an average of three to five. Occasionally a single perithecium is found. The ascospores are elongate-ellipsoidal and bicellular. The perithecia can survive as long as 14 months at temperatures of -15 to -18 C. The stem-canker fungus is known to survive in overwintered soybean stems (2,6,17,51). Perithecia with ascospores are produced during the late spring and summer.

Infection of soybean plants by DPC has been postulated to be either systemic or local and latent (2,28,50). Natural infection has never been observed before the plants are at least 62 days old (2). Crall (7) and Hildebrand (16) found perithecia of DPC on infected plants during the growing season and postulated that they might provide inoculum for infections late in the season, a view supported by Dunleavy (9). Other workers have reported that DPC perithecia are found only on overwintered soybean stems (2,6,51). Hilty (18) found perithecia on infected stem tissue in Tennessee as early as mid-February, with ascospores being detected by mid-March. The ascospores may serve as primary inoculum in the spring and summer, although this role has not been confirmed.

Although the stem canker disease cycle is not fully understood, plants are probably infected by rain-splashed propagules of the pathogen during early vegetative growth stages; however, disease symptoms develop only after the host's reproductive cycle begins, usually around pod set, growth stage R3 (39). Therefore, 1-2 months may pass between infection and symptom development. The belief that the stem-canker fungus can live in the soil for long periods of time has been expressed by some plant pathologists (14). In view of the appearance of stem canker in some fields that had not been planted previously to soybeans (15), it appears that the pathogen is either soil-borne or has hosts other than soybean. Several workers have examined the possibility of alternative hosts for DPC. Roy and Miller (43) found D. phaseolorum on cotton (Gossypium hirsutum L.) that produced stem cankers when inoculated to soybean. Hildebrand (16) was unsuccessful in establishing infections in several weed species in Canada. The saprophytic nature it exhibits in its ability to grow on plant material in the laboratory indicates that it may be able to survive in soil containing plant refuse. There is ample evidence that the organism can be found on seed although seed from even the most severely affected southern fields typically does not exceed 5% detectable infestation (3). The northern stem canker organism has been reported at frequencies exceeding 20% from seed of infected plants (20).

Clarification of the requirements for ascospore discharge and dissemination is needed to determine when infection occurs. Studies on Diaporthe stem canker of soybean were made in Iowa by Crall (7). Perithecia and mature ascospores were found infrequently on infected plants, providing possible means of secondary spread of the pathogen.

Average daily mean temperatures below 21 C favored the development of stem canker in the field, whereas 16 C was optimum in greenhouse tests. Typically, neither pycnidia nor perithecia are observed on infected plants during the summer season, although Krausz and Fortnum (30) and Hildebrand (16) observed perithecia during the growing season. Backman et al. (3) observed that perithecia usually developed during late winter (Feb.-Mar.) in the southeastern United States, and that ascospore release typically began during late April and continued into June. These ascospores were responsible for primary infections.

Ascospore Dissemination

Ascospores are in a sticky gelatinous matrix and are dispersed primarily by splashing rain. Wind-driven rain not only serves as an aid in dispersal but also supplies the necessary moisture for infection. Infections occur when the spores land on moist soybean leaves.

Several studies correlating temperature and humidity with infection have been conducted with other pyrenomycetous fungi. Holmes and Rich (21), in working with ascospores of Physalospora obtusa on apples, triggered spore discharge by simulating rainfall, but they found no consistent relationship between the amount of rainfall and the intensity of discharge. In addition, spores were most commonly released at temperatures from 6-16 C. Temperature was not a critical factor in determining whether or not spores were discharged. Their study further showed that the number of hours of 100 percent relative humidity within a 24 hour period was significantly correlated with the number of spores trapped in that period.

Linderman (33) found that forcible ejection of the ascospores of Calonectria spp. on azalea leaves appeared to occur at humidities ranging from 17-100 percent as a result of a change from a higher to a lower RH. High RH and probably even free water were necessary for perithecium formation and maturation. If these conditions persisted, ascospores would ooze from most of the perithecia. However, a slight decrease in RH when the perithecia are mature apparently induced some physiological changes that cause the ascospores to be forcibly discharged into the air.

Rowe & Beute (42), carried out a similar study of Cylindrocladium crotalariae (Loos) Bell and Sobers on peanuts. They observed that the development and ejection of ascospores occurred readily between 20-30 C and optimally at about 25 C. Ascospore ejection was stimulated by a reduction in relative humidity, but was not affected by raising the humidity. Gadoury (11) studied the ascigerous state of Venturia inaequalis, the causal agent of apple scab, to determine the effects of temperature on the development of pseudothecia. He found that the maturation rate of ascospores was directly proportional to temperatures ranging from 6-20 C.

The potential destructiveness of DPC is great. Because the disease is potentially destructive in Tennessee and little research about the effects of temperature and humidity on the development of the ascospores has been done, this study was undertaken.

II. MATERIALS AND METHODS

Isolation, Source and Maintenance of Culture Material

Isolates of Diaporthe phaseolorum var. caulivora used in this study were originally obtained from cankers on diseased Essex soybean plants obtained at the University of Tennessee, Knoxville. Cultures were maintained on petri plates of potato dextrose agar (PDA) placed at room temperature.

Relationship of Temperature on Perithecial Development

Healthy Essex soybean stems were collected from the field, cut into 4.0 cm long sections, and then surface-sterilized in 10 percent sodium hypochlorite for five minutes, followed by five minutes in distilled water. The stem segments were then further sterilized by placing them under a fume hood in a desiccator containing 5.0 ml propylene oxide. The stems were removed after 48 hours and partially submerged in warm water agar in 9-cm diameter plastic petri plates just prior to solidification (42). Two stems were placed in each of 72 petri plates. Each stem was then inoculated by placing a 5.0 mm-diameter plug of PDA with mycelium of Diaporthe phaseolorum var. caulivora on the center of each stem. The cultures were incubated at room temperature for 48 hours, then sealed with Parafilm to prevent drying. Twelve plates per temperature were placed in a series of incubators set at 15, 20, 22, 24, 25 and 30 C. The number of perithecia formed on aerial portions of each stem segment was

counted after three weeks incubation. The experiment was repeated twice. Duncan's Multiple Range test and a regression analysis were used in the analysis of the data.

Growth of Diaporthe Phaseolorum var. Caulivora in Culture

Isolates of Diaporthe phaseolorum var. caulivora were maintained on petri plates of PDA. Five-mm discs from the edges of cultures were obtained with a cork borer. These discs were then lifted with a transfer needle and transferred to the centers of plates of PDA. Fourteen plates, at each temperature, were placed randomly in a series of incubators set at 20, 22, 24 and 26 deg C. The diameter of the mycelia was measured at both day 3 and day 6 by taking the average of the two widest measurements of each colony. Duncan's Multiple Range test and a regression analysis were used in the analysis of the data.

Relationship of Humidity on Ascospore Discharge

Sterile soybean stem segments were partially submerged in water agar and inoculated with DPC as previously described. Ten plates were incubated at room temperature for three weeks to allow for perithecial development, and then placed in a sealed plexiglass chamber. The temperature in the chamber was held between 22-23 C and the relative humidity of the chamber was varied from 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 percent. An 11" X 14" X 2" aluminum pan filled with a glycerine-water mixture was placed in the center of the plexiglass chamber. Relative humidities were maintained by varying the

concentrations of this mixture as described by Johnson (25). A recording hygrometer was placed in the approximate center of the chamber to measure the humidity.

Microscope slides, coated on one side with a thin film of silica, were attached to the lids of petri dishes and suspended above the perithecia at varying distances from 0.5 cm to 5.0 cm, to observe possible forced ejection of the ascospores. Slides were microscopically observed for the presence of ascospores at one minute intervals, for 10 minutes at each humidity.

Droplet formation and oozing of the ascospores by the perithecia were also observed. The times of ascospore release were confirmed by microscopic observation of droplets which contained ascospores.

Histological Studies

To obtain a better understanding of the development of DPC within the host, histological studies were undertaken. Sterile soybean stem segments, one cm long, were partially submerged in water agar and inoculated with Diaporthe phaseolorum var. caulivora, as described in the previous experiments. Cultures were incubated at room temperature. Stem segments were taken from the cultures at two-day intervals beginning five days after inoculation.

The stem tissues were fixed in a solution of formalin-acetic acid-ethanol (FAA) (23). After a period of 24 hours, the stem tissues were run through a graded series of increasing ethanol concentrations from 10% to 40%. Dehydration was completed by using a series of increasing tertiary-butyl alcohol concentrations starting at 50% concentration and

finishing with pure tertiary-butyl alcohol according to Jenson (23). The specimens were embedded in paraffin (Tissue Prep 56.5 C, Fisher Scientific Co.) and sectioned at 10 μ m. The sections were mounted on slides and stained with a triple stain consisting of an aqueous solution of Safranin (1 g/l) and a mixture of aniline blue (5 g/l) and orange G (20 g/l) in a 0.1 M citrate buffer (pH 3.0) as described by McDaniel et al. (35). Cover glasses were affixed and sealed with resin.

III. RESULTS

Effect of Temperature on Perithecial Development

Perithecial development occurred both as groups in a stroma and individually within 3 weeks at 20, 22, 24, 25 and 26 C. Development did not occur at 15 C or 30 C (Table 1). Growth at the four temperatures denoted as b were not significantly different from each other when tested at the 0.05 level of significance. Growth at the four temperatures denoted as c were also found not to be significantly different. This suggests that the only significant difference is between 22 and 26 C, with growth at 22 C significantly greater than growth at 26 C. The optimum temperature for development of the perithecia in culture was 22 C.

Regression analysis was performed to determine the percent variance in growth of perithecial development predictable from differences in temperature. At the 0.05 level of significance, the percent variance accounted for was significant, although only four percent of the variance could be predicted. However, this is not a high enough percentage to be useful in predicting the optimum conditions for disease development.

Growth of *Diaporthe Phaseolorum* var. *Caulivora* in Culture

Growth diameter in the cultures increased with increasing temperatures from 20-26 C (Figure A). No significant differences in colony growth were noted between 20 and 22 C. However, growth at these

TABLE 1. Effect of temperature on perithecial development of Diaporthe
phaseolorum var. caulivora

Temperature C	$\bar{x}$ perithecia/inoculation
15	0a ¹
20	16.3bc
22	19.8c
24	12.8bc
25	10.8bc
26	7.4b
30	0a

¹ Numbers are the means of 12 replicates. Numbers followed by a common letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

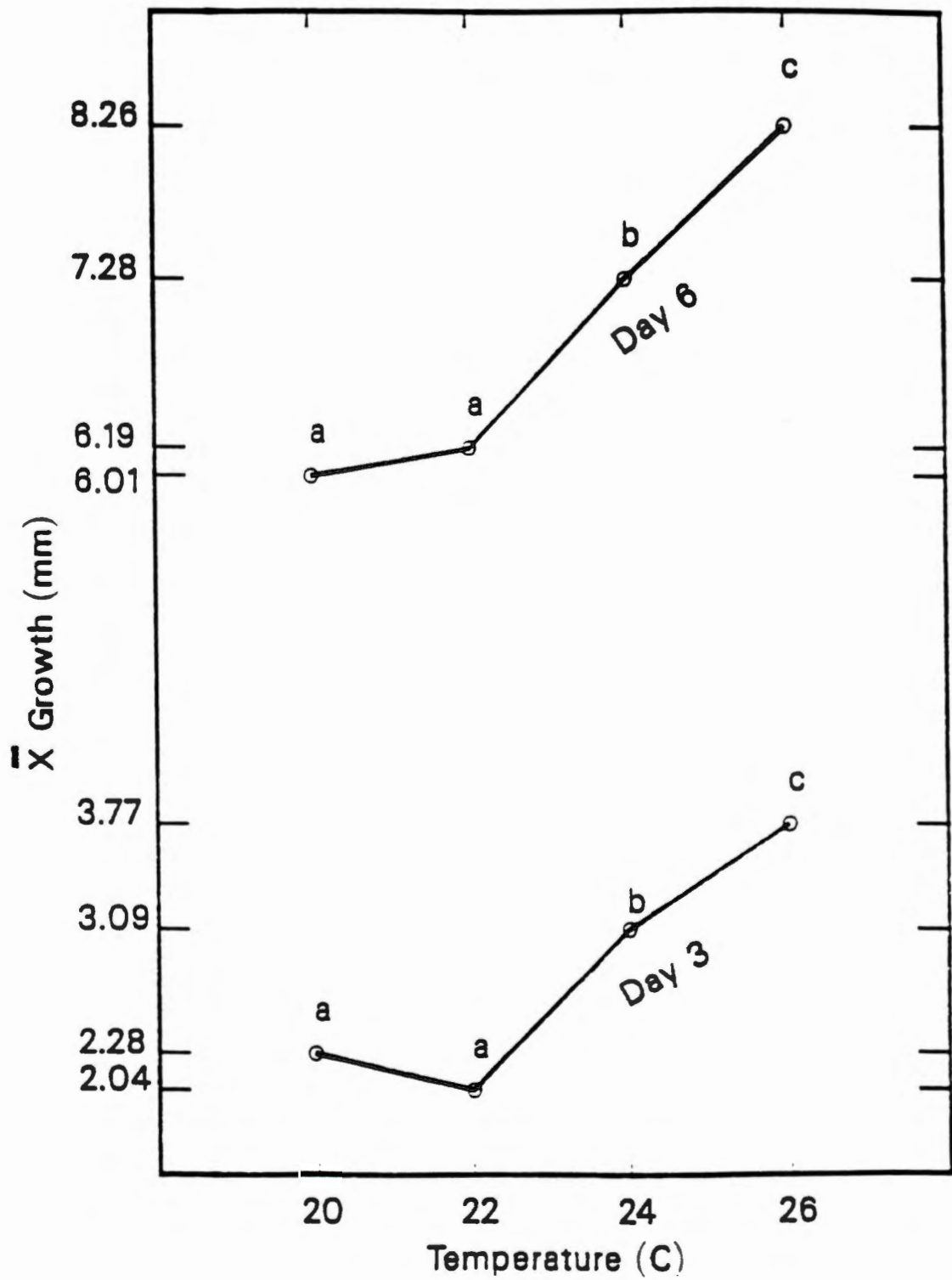


FIGURE 1. Growth of *Diaporthe Phaseolorum* Var. *Caulivora* in Culture

temperatures was significantly lower than growth at 24 C, which in turn was significantly lower than 26 C. These relationships were similar for both day three and day six.

Effect of Humidity on Ascospore Discharge

The release of ascospores from 3-week old perithecia occurred within five minutes at relative humidities of 70-100% at 22-23 C. At 60 percent relative humidity, ascospore release occurred in 15 minutes. However, when the relative humidity was lowered to 55 percent and below, no discharge occurred. Numerous attempts were made to determine the optimum relative humidity for ascospore discharge. The general conclusion from these tests, which involved relative humidities from 20-100%, was that the ascospores were discharged from perithecia at all relative humidities above 55%, but generally more were discharged at relative humidities of 70-100%. It could not be determined whether these differences were significant due to the small number of observations at each humidity. No spores were trapped on the suspended microscope slides. The ascospores were seen exuding from perithecia as golden droplets.

Histological Studies

Within 5-7 days after inoculating sterilized soybean stems with DPC, mycelia aggregated into perithecial initials (Plate 1, Figure A). These initial cells gave rise to hyphae which formed the base of the developing perithecium. The enveloping hyphae formed two to three regular layers of cells within the developing perithecium (Plate 1, Figure B). At this early stage in development, perithecial hairs began to grow from the

Plate 1. Perithecial ontogeny and ascospore discharge were followed histologically.

Figure A. Mycelia aggregated into perithecial initials on the surface of colonized soybean stems five to seven days after inoculations. (x173)

Figure B. Perithecial initials then began to enlarge and the interior filled with rows of parenchymatous cells. (x103)

Figure C. By the 14th day, the perithecial cavity began to form by disintegration of the enlarged, parenchymatous cells and asci began to elongate into the cavity from the darkly stained hymenium layer. (x103)

Figure D. Eighteen days after inoculation, the perithecial cavity was completely open and the ostiole had formed. (x111)

Figure E. Mature asci containing ascospores were observed in about three weeks after inoculation. (x220)

Figure F. A single ascus, broken loose from the hymenium layer was found lodged in the ostiole. (x220)

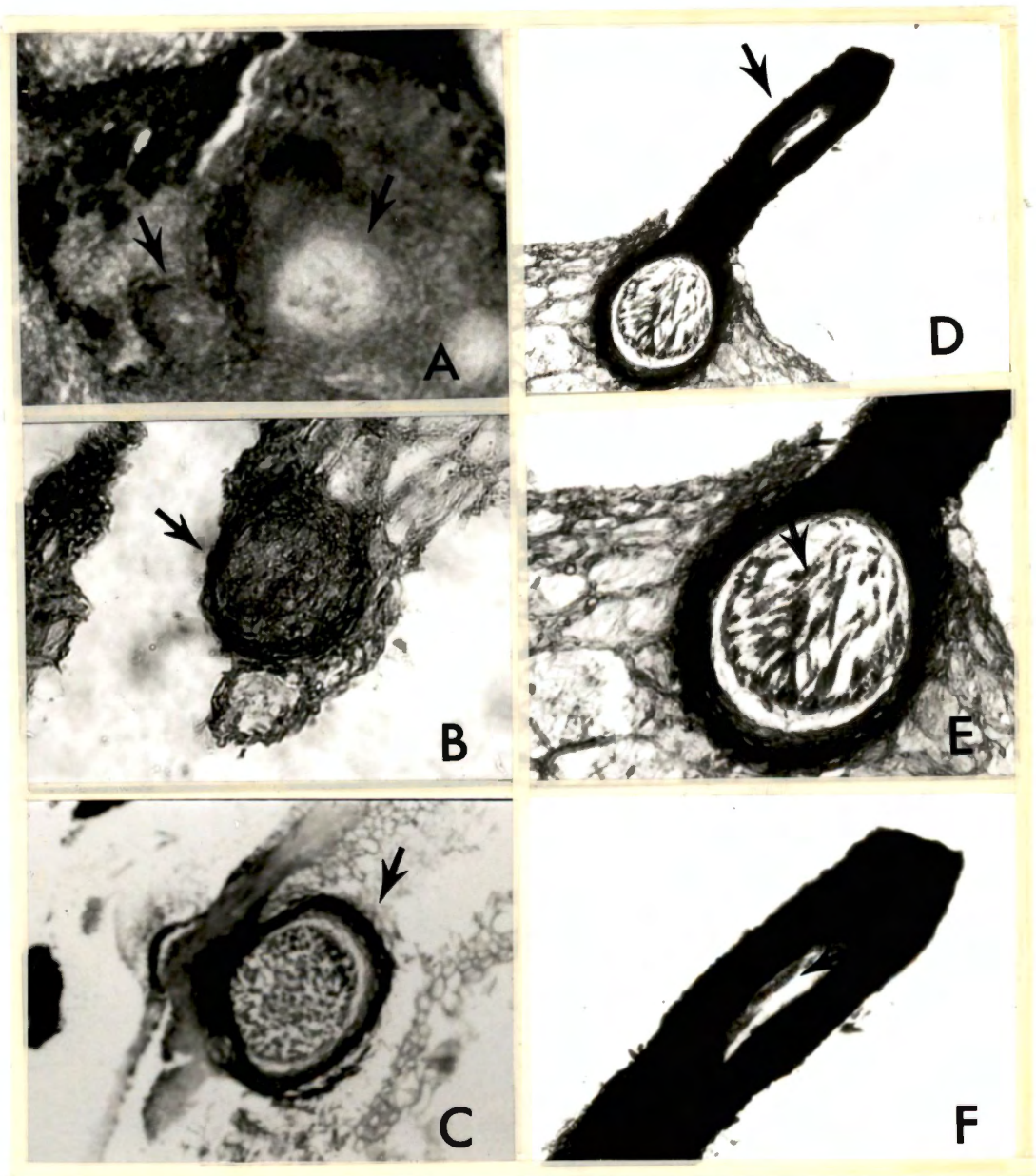


PLATE 1

outermost layer of cells. Continued divisions and enlargement of the cells eventually produced the mature perithecia. The developing cells of the central region were thin-walled and pseudoparenchymatous (Plate 1, Figure C). In perithecia containing mature asci, the outer region consisted of one to two layers that were thicker-walled than the inner cells and were dark brown in color (Plate 1, Figure D). These cells retained a pseudoparenchymatous appearance over most of the perithecium except in the upper ostiole region where they were rectangular in outline (Plate 1, Figure C).

Development of the ostiole and neck occurred in the apical portion of the perithecium (Plate 1, Figure C,D). The ostiole resulted from the lysis of cells in this region and became continuous with the developing centrum cavity. In sectioned material, periphyses that lined the ostiole were scarce. The outer rectangular cells elongated above the ostiole forming a hollow beak-like structure 2-3 times the length of the mature perithecium through which ascospores were released in a single column (Plate 1, Figure D). The formation of the centrum cavity resulted from the breakdown of pseudoparenchyma cells in advance of the developing ascogenous hyphae and asci (Plate 1, Figure C). Hymenial paraphyses were not observed during development of the centrum.

Developing asci were observed (Plate 1, Figure C,D). Mature asci containing eight ascospores were observed about 3 weeks after inoculation (Plate 1, Figure E). The asci were released from the perithecium after 3 weeks of development (Plate 1, Figure F).

IV. DISCUSSION

Seasonal variation in stem canker outbreaks has been attributed in part to yearly differences in environmental conditions (4,40,41). Results of this study indicate that temperature is an important environmental factor influencing perithecial development of DPC. The range of temperatures for perithecial development was broad (>15 C to <30 C). High temperatures (>30 C) and temperatures below 15 C prevented development of perithecia by this pathogen. Although stem canker is an important disease in the Southeast, little is known about factors influencing the development of the perithecia. Ploetz (40) reported that infection of soybean seedlings by southern Diaporthe phaseolorum (Cke & Ell.) Sacc. occurred within a wide range of temperatures (10-34 C), with maximum levels of infection by ascospores and alpha-conidia at 28 and 34 C; lower levels of infection occurred at 10, 16 and 22 C. Also, extremely high temperatures in the soybean canopy (>40 C) would stop infection by this pathogen from occurring.

The effect of temperature on the growth of DPC in culture was also studied to compare with the development of the disease in the field. The growth diameter of the cultures increased with increasing temperatures from 20-26 C. Similar experiments with DPC are not known by this author, however, temperature is a factor known to influence host infections (48,40,46).

In this study, relative humidities above 55% were also found to stimulate release of ascospores of DPC. Lehman (33) observed that plant infection and the dissemination of D. phaseolorum var. sojae inoculum

were strongly dependent on high humidity and that subsequent seed infection was much more abundant during rainy summers. Wilcox et al. (52), obtained similar results. They concluded that high temperature and humidity in September and early October probably favor the development of D. phaseolorum var. sojae and other fungi in seeds. Their results agree with Kmetz et al. (29) and their studies of Phomopsis sp. They indicate that optimum conditions for seed infection are high levels of rain, temperature and relative humidity. Spilker et al. (46), reported similar results in their study on the effects of humidity and temperature on the reduction of soybean seed quality by Phomopsis sp. They found that the percentage of moldy seeds was highest at high (33 C day, 24 C night) temperature and high (90%) relative humidity combinations. Recently, Damicone et al. (8) reported on the relationship of continuous and discontinuous free moisture to stem canker incidence. They found that disease incidence and severity and length of the incubation period were related more to the total moisture duration than to the type.

The ejection of spores, as reported for other ascomycetes, depends upon several interrelated factors including the amount and duration of rainfall, temperature and relative humidity, as well as moisture content of the fungus stroma (1,21,22,33,36,46,49).

Rowe (42) found that the development and ejection of ascospores of Calonectria crotalariae on peanuts occurred readily between 20-30 C and optimally at about 25 C. This follows closely with this author's observations of DPC with ascospores. Rowe concluded that discharge of the ascospores could involve the development of hydrostatic pressure within the perithecial cavity. A sudden drop in humidity may cause

desiccation and contraction of the large parenchymatous cells comprising the outer wall of the perithecium.

Ingold and Vivienne (22) noted similar results with their work on Sordaria fumicola (Rob.) Ces. and de Not. Ascospore ejection was also stimulated upon a reduction in atmospheric humidity. Their explanation for this was that a reduction in humidity was correlated with air movement favoring dispersal.

The histological findings follow closely with those of Jenson (24) in his work with the histological development of Diaporthe phaseolorum var. sojae in culture. He reported that perithecial development takes 20-25 days under laboratory culture conditions and he also reported the presence of paraphyses in the perithecium. Traditionally, the Diaporthe type centrum has been depicted as lacking paraphyses. Lutrell (34) also supports the observance of paraphyses. Jenson (24) did not, however, observe forcible ejection of the ascospores. Research is still needed to determine the effect of various temperatures at various humidities in addition to other factors (39,44,45) that may influence the development of this disease.

LIST OF REFERENCES

LIST OF REFERENCES

1. Athow, K. L. and R. M. Caldwell. 1954. A comparative study of Diaporthe stem canker and pod and stem blight of soybeans. *Phytopathology* 44: 319-325.
2. Athow, K. L. and F. A. Laviolette. 1973. Pod protection effects on soybean seed germination and infection with Diaporthe phaseolorum var. sojae and other microorganisms. *Phytopathology* 63: 1021-1025.
3. Backman, P. A., M. A. Crawford, J. White, D. L. Thurlow and L. A. Smith. 1981. Soybean stem canker: a serious disease in Alabama. *Highlights Agriculture Research* 28 (4): 6.
4. Backman, P. A., D. B. Weaver and G. Morgan-Jones. 1985. Soybean stem canker: An emerging disease problem. *Plant Disease* 69: 641-647.
5. Crall, J. M. 1950. Soybean diseases in Iowa in 1949. *Plant Disease Reporter* 34: 96-97.
6. Crall, J. M. 1951. Soybean diseases in Iowa in 1950. *Plant Disease Reporter* 35: 320-321.
7. Crall, J. M. 1956. Observations on the occurrence of soybean stem canker. (Abstr.) *Phytopathology* 46: 10.
8. Damicone, J. P., G. T. Berggren and J. P. Snow. 1987. Effect of free moisture on soybean stem canker development. *Phytopathology* 77: 1568-1572.
9. Dunleavy, J. M. 1954. Soybean diseases in Iowa in 1953. *Plant Disease Reporter* 38: 89-90.
10. Frosheiser, F. I. 1957. Studies on the etiology and epidemiology of Diaporthe phaseolorum var. caulivora, the cause of stem canker in soybeans. *Phytopathology* 47: 87-94.
11. Gadoury, D. M. and W. E. MacHardy. 1982. A model to estimate the maturity of ascospores of Venturia inaequalis. *Phytopathology* 72: 901-904.
12. Harter, L. L. 1917. Pod blight of the lima bean caused by Diaporthe phaseolorum. *J. Ag. Research* 11: 473-504.
13. Harter, L. L. and Ethel C. Field. 1912. Diaporthe, the ascogenous form of sweet potato dry rot. *Phytopathology* 2: 121-134.

14. Hildebrand, A. A. 1952. Stem canker, a disease of increasing importance on soybeans in Ontario. *Soybean Digest* 12 (9): 12-15.
15. Hildebrand, A. A. 1954. Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybean. *Plant Disease Reporter* 38: 640-646.
16. Hildebrand, A. A. 1956. Observations on stem canker and pod and stem blight of soybeans in Ontario. *Can. J. Botany* 34: 577-599.
17. Hilty, J. W. 1981. Soybean stem canker, a major disease in 1981. *Tennessee Farm and Home Science* 120: 16-17.
18. Hilty, J. W. 1982. Personal Communication. University of TN, Knoxville.
19. Hobbs, T. W. and D. V. Phillips. 1985. Identification of Diaporthe and Phomopsis isolates from soybean. (Abstr.) *Phytopathology* 72: 807-809.
20. Hobbs, T. W., A. F. Schmitthenner, C. Ellett and R. E. Hite. 1981. Top dieback of soybean caused by Diaporthe phaseolorum var. caulivora. *Phytopathology* 72: 807-809.
21. Holmes, J. and A. E. Rich. 1970. Factors affecting release and dissemination of Physalospora obtusa spores in a New Hampshire apple orchard. *Phytopathology* 60: 126-132.
22. Ingold, C. T., and Vivienne J. Dring. 1952. An analysis of spore discharge in Sordaria. *Ann. Bot. n.s.* 21: 465-477.
23. Jenson, W. A. 1962. Botanical histochemistry, principles and practice. W. H. Freeman and Co., San Francisco. 408 pp.
24. Jenson, J. D. 1983. The development of Diaporthe phaseolorum var. sojae in culture. *Mycologia* 75: 1074-1091.
25. Johnson, C. G. 1940. The maintenance of high atmospheric humidities for entomological work with glycerol-water mixture. *Ann. Appl. Biol.* 27: 295-299.
26. Keeling, B. L. 1985. Soybean cultivar reactions to soybean stem canker caused by Diaporthe phaseolorum var. caulivora and pathogenic variation among isolates. *Plant Disease* 69: 132-133.
27. Keeling, B. L. 1984. Evidence for physiologic specialization in Diaporthe phaseolorum var. caulivora. *J. Miss. Acad. Sci. Suppl.* 29: 5.
28. Kilpatrick, R. A. 1957. Fungi associated with flowers, pods and seeds of soybeans. *Phytopathology* 47: 131-135.

29. Kmetz, K. T., C. W. Ellett and A. F. Schmitthenner. 1979. Soybean decay: sources of inoculum and nature of infection. *Phytopathology* 69: 798-801.
30. Krausz, J. P. and B. A. Fortnum. 1983. An epiphytotic of Diaporthe stem canker of soybean in South Carolina. *Plant Disease* 67: 1128-1129.
31. Kulick, M. M. 1984. Symptomless infection, persistence and production of pycnidia in host and non-host plants by Phomopsis batatatae, Phomopsis phaseoli, and Phomopsis sojae and the taxonomic implications. *Mycologia* 76: 274-291.
32. Lehman, S. G. 1923. Pod and stem blight of soybean. *Ann. Missouri Botan. Garden* 10: 111-178.
33. Lindermann, R. G. 1974. Ascospore discharge from perithecia of Calonectria theas, C. crotalariae, and C. kytensis. *Phytopathology* 64: 567-569.
34. Luttrell, E. S. 1965. Paraphysoids, pseudoparaphyses and apical paraphyses. *Trans. Brit. Mycol. Soc.* 48: 135-144.
35. McDaniel, J. K., B. V. Conger and E. T. Graham. 1982. A histological study of tissue cultures of Dactylis glomerata. *L. Protoplasma* 110: 121-128.
36. McGee, D. C. and J. A. Biddle. 1987. Seedborne Diaporthe phaseolorum var. caulivora in Iowa and its relationship to soybean stem canker in the Southern United States. *Plant Disease* 71: 620-622.
37. Morgan-Jones, G. 1985. The Diaporthe-Phomopsis complex on soybean: morphology. In: *Proceedings of the Conference on the Diaporthe-Phomopsis Disease Complex on Soybean*. Agriculture Research Service U. S. Dept. Agric. pp. 1-7.
38. Ploetz, R. C. and F. M. Shokes. 1985. Current status of soybean stem canker in the Southeastern United States. (Abstr.) *Phytopathology* 75: 503.
39. Ploetz, R. C. and F. M. Shokes. 1985. Soybean stem canker incited by ascospores and conidia of the fungus causing the disease in the Southeastern United States. *Plant Disease* 69: 990-992.
40. Ploetz, R. C. and F. M. Shokes. 1987. Factors influencing infection of soybean seedlings by southern Diaporthe phaseolorum. *Phytopathology* 77: 786-790.
41. Rothrock, C. S., T. W. Hobbs and D. V. Phillips. 1985. Effects of tillage and cropping systems on incidence and severity of southern stem canker of soybean. *Phytopathology* 75: 1156-1159.

42. Rowe, R. C. and M. K. Beute. 1975. Ascospore formation and discharge by Calonectria crotalaria. *Phytopathology* 65: 393-398.
43. Roy, K. W. and W. A. Miller. 1983. Soybean stem canker incited by isolates of Diaporthe and Phomopsis spp. from cotton in Mississippi. *Plant Disease* 67: 135-137.
44. Smith, C. M., A. E. Davis and M. J. Fielding. 1985. Environmental factors affecting development of Diaporthe stem canker in soybean seedlings. (Abstr.) *South. Soybean Disease Workers Proc.* 12: 81.
45. Smith, E. 1986. Epidemiology of Diaporthe phaseolorum var. caulivora in southern soybeans. (Abstr.) *South. Soybean Disease Workers Proc.* 13: 16.
46. Spilker, D. A., A. F. Schmitthenner and C. W. Ellett. 1981. Effects of humidity, temperature, fertility and cultivar on the reduction of soybean seed quality by Phomopsis sp. *Phytopathology* 71: 1027-1029.
47. Threinen, J. T., T. Kommedall and R. J. Klug. 1959. Hybridization between radiation-induced mutants of two varieties of Diaporthe phaseolorum. *Phytopathology* 49: 797-801.
48. Timnick, M. B., V. G. Lilly and H. L. Barnett. 1951. Factors affecting sporulation of Diaporthe phaseolorum var. batatatis from soybean. *Phytopathology* 41: 327-336.
49. Tschanz, A. T., R. K. Horst and Paul E. Nelson. 1975. Ecological aspects of ascospore discharge in Gibberella zeae. *Phytopathology* 65: 597-599.
50. Wehmeyer, Lewis E. 1933. The genus Diaporthe nitschke and its segregates. University of Michigan press, Ann Arbor. 349 pp.
51. Welch, A. N. and Joseph C. Gilman. 1948. Hetero- and homothallic types of Diaporthe on soybeans. *Phytopathology* 38: 629-637.
52. Wilcox, J. R., R. A. Laviolette and K. L. Athow. 1974. Deterioration of soybean seed quality associated with harvest delay. *Plant Disease Rep.* 58: 130-133.

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