# Soybean Stem Canker Incited by Ascospores and Conidia of the Fungus Causing the Disease in the Southeastern United States

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**ABSTRACT**

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In artificial inoculation experiments, ascospores or conidia of the fungus causing soybean stem canker in the southeastern United States infected a resistant cultivar and three susceptible cultivars of soybean. Typical symptoms of the disease developed on inoculated plants of susceptible cultivars 50–80 days after inoculation.

Additional key words: *Diaporthe phaseolorum* var. *caulivora*, southern *D. phaseolorum*

Soybean stem canker is an economically important disease in the southeastern United States that is responsible, periodically, for large reductions in yield (9). 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 [5]). Therefore, 1–2 mo may pass between infection and symptom development.

Pathologists studying the disease have used Crall's (4) toothpick method when artificially inducing the disease. This technique involves growing isolates of the pathogen on toothpicks. Colonized toothpicks are then inserted through stems of young soybean plants, and the wounds are sealed with petroleum jelly. In compatible combinations, disease symptoms develop about 10 days after inoculation. Although rapid development of symptoms is an advantage of this technique, it also has several disadvantages including 1) high labor intensity; 2) the unnatural manner in which the pathogen is introduced into the host, bridging its natural defenses; and 3) use of an unnatural form of inoculum. Underscoring the latter criticism is the fact that cultivars of soybean known to be highly resistant in the field may develop typical symptoms of the disease when toothpick-inoculated (R. C. Ploetz and F. M. Shokes, *unpublished*).

A stem canker of soybeans caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell has been known in the midwestern United States since the 1940s (1). In general, the morphology of the pathogens and symptomatology of the midwestern and southeastern stem cankers are similar. However, there is increasing evidence that the fungi causing the two diseases are distinct. Hobbs and Phillips (7) 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 *D. phaseolorum* var. *caulivora* 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." Morgan-Jones and Backman (10,11) cited differences between northern and southern stem canker isolates in color, growth rate, temperature relations, stroma size, and perithecium and ascospore morphology. They suggested that a distinct variety of *D. phaseolorum* may cause soybean stem canker in the southeastern United States. Finally, in work in our laboratory, vegetative compatibility groups that exist among southern isolates have not been found among isolates of *D. phaseolorum* var. *caulivora* (R. C. Ploetz and F. M. Shokes, *unpublished*). In addition, we have noted growth inhibition of isolates of *D. phaseolorum* var. *caulivora* on a medium selective for the growth of southern isolates (12). Although *D. phaseolorum* var. *caulivora* and the organism causing stem canker in the Southeast are probably closely related, the two fungi can be distinguished by several objective criteria. To recognize these differences, we refer to the latter pathogen as southern *D. phaseolorum*.

This work was undertaken to examine the role of conidia and ascospores of southern *D. phaseolorum* in the disease cycle of soybean stem canker in the southeastern United States.

## MATERIALS AND METHODS

All plants used in the following experiments were grown in Metro Mix 220 (Grace Horticultural Products, W. R. Grace & Co., Cambridge, MA) in plastic pots either 15 cm (experiments 1 and 3) or 30 cm in diameter (experiment 2). Plants in experiment 1 were fertilized with 20-20-20 granular fertilizer as needed, and plants in experiments 2 and 3 were treated, as seeds, with Nitragin soybean inoculant (Nitragin Co., Milwaukee, WI) and fertilized with 3-9-18 and chelated iron fertilizers as needed. Plants were watered by hand. All three experiments were initiated in glass greenhouses. Experiment 3 was kept in the greenhouse until its completion, but experiments 1 and 2 were moved outside about 1.5 mo after inoculation because of extreme heat in the greenhouse.

Plants in all experiments were watered

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thoroughly, and foliage was moistened before inoculation. Spore suspensions were used for all inoculations. Plants in experiments 1 and 2 were inoculated using a shaker with a perforated aluminum foil cap, and plants in experiment 3 were inoculated using a Sure Shot sprayer (Milwaukee Sprayer, Milwaukee, WI) pressurized with $CO_2$. Immediately after inoculation, plants were covered with plastic bags supported by bamboo stakes and secured at the pot base with rubber bands. Bags were removed in experiments 1 and 2 about 12 hr after inoculation; bags in experiment 3 were removed 36 hr after inoculation. Before inoculation, it was necessary to keep plants in experiments 1 and 3 under 14 hr of daily supplemental fluorescent light to retard flowering; supplemental light was discontinued after inoculation.

Natural inoculum was obtained from fruiting bodies on soybean debris recovered from a field devastated by stem canker in 1983. Debris was incubated over moistened paper towels in the laboratory for 1–4 wk before collection of spores. Artificially produced inoculum was obtained from 4- to 6-wk-old Difco potato-dextrose agar (PDA) cultures of isolates of southern *D. phaseolorum*. Conidia or ascospores from soybean debris or PDA were obtained by excising pycnidia or perithecia, respectively, from the appropriate source and macerating the fruiting bodies in deionized water to liberate spores. The preparations were strained through several layers of cheesecloth before spore concentrations were determined with a hemacytometer.

Tissue isolations were made from inoculated and uninoculated plants during all experiments. Symptomatic and asymptomatic tissue was excised from stems and leaf petioles, surface-disinfested with 0.5% NaOCl for 1–2 min, rinsed twice in sterile deionized water, blotted dry on sterile paper towels, and placed on Phillips' (12) medium. Tissue was incubated without light at about 25 C for 3–7 days before it was observed for growth of southern *D. phaseolorum*. Phillips' (12) medium promotes growth of southern *D. phaseolorum* but inhibits the growth of closely related fungi (i.e., *D. phaseolorum* var. *caulivora*, *D. phaseolorum* var. *sojae* (Lehman) Wehm., and *Phomopsis longicolla* Hobbs apud Hobbs et al).

**Experiment 1.** Natural or artificially produced ascospores of the pathogen were used to inoculate soybean plants (susceptible cultivar Hutton) of different growth stages (V8, V9, V10, and V11 [5]). Two plants of each age were inoculated for each ascospore treatment; spore concentrations for each treatment were about $10^5$/ml. Two uninoculated plants of each growth stage were misted with deionized water and used as controls.

**Experiment 2.** Plants of a resistant cultivar (Braxton) and a susceptible cultivar (RA 604) were inoculated at one of four growth stages (V1, V3, V6, or V9) with natural or artificially produced conidia or ascospores of the pathogen. Three pots with two plants each were used for each cultivar × inoculum type × growth stage combination. For each cultivar, three pots with two plants each were misted with deionized water for uninoculated controls. Plants at stage V1 were inoculated with natural ascospores (about $10^5$/ml), whereas V3, V6, and V9 plants were inoculated with artificially produced ascospores ($<10^4$, $10^5$, and $10^5$/ml, respectively). V1 and V6 plants were inoculated with natural conidia ($<10^4$ and $10^5$/ml, respectively), and V3 and V9 plants were inoculated with artificially produced conidia ($10^6$ and $10^5$/ml, respectively).

**Experiment 3.** Plants of three susceptible cultivars (Bragg, Hutton, and RA 604) were inoculated with different concentrations of artificially produced conidia (Table 1). For each cultivar, three plants in each of two pots were inoculated for each inoculum concentration; three uninoculated plants sprayed with deionized water were used as controls for each cultivar. Bragg plants were at growth stage V5, RA 604 plants at V3, and Hutton plants at V6 when inoculated.

## RESULTS AND DISCUSSION

Stem canker symptoms developed on plants inoculated with ascospores or conidia in each of three experiments (Table 1). Symptoms did not develop on uninoculated plants. The pathogen was isolated routinely from stem lesions and asymptomatic tissue from inoculated susceptible (Bragg, Hutton, and RA 604) and resistant (Braxton) plants but was not recovered from uninoculated plants.

The source of ascospores or conidia (naturally or artificially produced) had no apparent effect on disease development. Ascospores and conidia of southern *D. phaseolorum* were commonly produced

**Table 1.** Disease development on soybeans inoculated with naturally or artificially produced ascospores or conidia of the fungus causing soybean stem canker in the southeastern United States

| Cultivar | Growth stage at inoculation | Type of inoculum[u] A | Type of inoculum[u] C | ID[v] | Incidence[w] | Symptoms detected (days)[x] |
|---|---|---|---|---|---|---|
| | | | Experiment 1 | | | |
| Hutton | V8 | n[y] | ··· | $10^5$ | 100 | 61 |
| Hutton | V9 | n | ··· | $10^5$ | 100 | 75 |
| Hutton | V10 | n | ··· | $10^5$ | 100 | 75 |
| Hutton | V11 | n | ··· | $10^5$ | 100 | 75 |
| Hutton | V8 | a[z] | ··· | $10^5$ | 100 | 75 |
| Hutton | V9 | a | ··· | $10^5$ | 100 | 75 |
| Hutton | V10 | a | ··· | $10^5$ | 100 | 75 |
| Hutton | V11 | a | ··· | $10^5$ | 100 | 75 |
| | | | Experiment 2 | | | |
| RA 604 | V1 | n | ··· | $10^5$ | 33 | 80 |
| Braxton | V1 | n | ··· | $10^5$ | 0 | – |
| RA 604 | V1 | ··· | n | $<10^4$ | 0 | – |
| Braxton | V1 | ··· | n | $<10^4$ | 0 | – |
| RA 604 | V3 | a | ··· | $<10^4$ | 33 | 66 |
| Braxton | V3 | a | ··· | $<10^4$ | 0 | – |
| RA 604 | V3 | ··· | a | $10^6$ | 100 | 66 |
| Braxton | V3 | ··· | a | $10^6$ | 0 | – |
| RA 604 | V6 | a | ··· | $10^5$ | 100 | 60 |
| Braxton | V6 | a | ··· | $10^5$ | 0 | – |
| RA 604 | V6 | ··· | n | $10^6$ | 100 | 60 |
| Braxton | V6 | ··· | n | $10^6$ | 0 | – |
| RA 604 | V9 | a | ··· | $10^5$ | 83 | 54 |
| Braxton | V9 | a | ··· | $10^5$ | 0 | – |
| RA 604 | V9 | ··· | a | $10^5$ | 100 | 54 |
| Braxton | V9 | ··· | a | $10^5$ | 17 | 54 |
| | | | Experiment 3 | | | |
| Bragg | V5 | ··· | a | $10^4$ | 0 | – |
| Bragg | V5 | ··· | a | $10^3$ | 0 | – |
| RA 604 | V3 | ··· | a | $10^4$ | 0 | – |
| RA 604 | V3 | ··· | a | $10^3$ | 33 | 50 |
| Hutton | V6 | ··· | a | 1 | 0 | – |
| Hutton | V6 | ··· | a | 10 | 0 | – |
| Hutton | V6 | ··· | a | $10^2$ | 0 | – |
| Hutton | V6 | ··· | a | $10^3$ | 17 | 50 |
| Hutton | V6 | ··· | a | $10^4$ | 0 | – |

[u] A = ascospores and C = conidia.
[v] Inoculum density: spores per milliliter.
[w] Percentage of plants that developed stem canker symptoms; symptoms were verified by isolation of the causal organism.
[x] Number of days after inoculation that symptoms were detected; – = no symptoms.
[y] Natural inoculum produced on cankered soybean stems.
[z] Inoculum artificially produced on potato-dextrose agar.

on cankered soybean stems recovered from the field after 5–10 days of incubation over moistened paper towels in the laboratory (23–28 C). Because similar environmental conditions occur in the field during periods when host infection occurs, it is possible that ascospores and conidia of the pathogen are produced concurrently in the field and that both participate in infection of the host in the spring.

Frosheiser (6) inoculated susceptible soybean cultivars in the greenhouse with ascospores or conidia of *D. phaseolorum* var. *caulivora* in Minnesota. Although he was able to reisolate the pathogen from plants inoculated with either type of spore ($\geqslant 3 \times 10^4$/ml), symptoms did not develop on inoculated plants. Inoculation of soybeans with ascospores of southern *D. phaseolorum* has been reported twice previously (13,14). Sciumbato and Keeling (13) described inoculating resistant and susceptible cultivars in the field with $1.5 \times 10^4$ ascospores per milliliter. Although symptom development occurred on susceptible plants in their work, they did not report the use of uninoculated controls needed to rule out infection from other sources of inoculum (i.e., natural inocula from infested soybean debris in the field or infested seed). Smith et al (14) reported symptom development on Hutton seedlings in growth chambers after inoculation with $10^5$ ascospores per milliliter. During their experiments, plants were misted for 1 day after inoculation, then incubated for 13 days under various moisture regimes. Plants kept under continuous mist for 14 days developed numerous small stem lesions, but plants kept under intermittent mist for 13 days developed few lesions. No lesions developed on plants incubated at a relative humidity of 50% for the last 13 days of the experiment. The pathogen was isolated from inoculated plants incubated under each of the moisture regimes but was not isolated from uninoculated plants.

In our experiments, plant surfaces were dry for at least 40 days after a moist incubation period of 12–36 hr after inoculation. The pathogen was commonly recovered from surface-disinfested tissue of these plants during isolations beginning 1 wk after inoculation. In the work of Smith et al (14), infection of plants kept under a "dry" regime (1 day of mist followed by 13 days of a relative humidity of 50%) was detected 14 days after inoculation. Therefore, infection in both cases probably occurred within 12–24 hr of inoculation. Additional work, however, needs to be conducted before determining the influence of free moisture on the infection of soybeans by this pathogen.

In our work, symptom development was detected only after the R2–R3 growth stages of the host, or 50–80 days after inoculation (Table 1). Symptoms were not detected on these plants as soon after inoculation as reported by Smith et al (14), but this was probably due to differences in the environments under which plants were incubated. In our studies, disease development was dependent on maturation of the host, but in their work, environmental stress (high moisture) was the probable cause of symptom development.

Although the influence of inoculum density on disease development was not evaluated per se in experiments 1 and 2, results from our experiments demonstrate a higher incidence of disease with increasing inoculum densities (Table 1). Inoculum densities of the pathogen may also affect the incidence and severity of the disease in the field. It has been suggested that other factors such as rainfall (3) or drought (i.e., water stress [2]) may also influence the occurrence of this disease.

Inoculation with spore suspensions, as done in this study, would not be as expedient a method as the toothpick technique for evaluating breeding lines of soybean for resistance to stem canker (8). However, the technique we have described would be useful in studies on the ecology and biology of the pathogen causing the disease in the Southeast.

**LITERATURE CITED**

1. Athow, K. L., and Caldwell, R. M. 1954. A comparative study of Diaporthe stem canker and pod and stem blight of soybeans. Phytopathology 44:319-325.
2. Backman, P. A., Crawford, M. A., White, J., Thurlow, D. L., and Smith, L. A. 1981. Soybean stem canker: A serious disease in Alabama. Highlights Agric. Res. 28(4):6.
3. Berggren, G. T., Snow, J. P., and McGawley, E. C. 1984. Distribution of soybean stem canker in Louisiana. (Abstr.) Phytopathology 74:872.
4. Crall, J. M. 1952. A toothpick tip method of inoculation. (Abstr.) Phytopathology 42:5-6.
5. Fehr, W. R., and Caviness, C. E. 1977. Stages of soybean development. Iowa Agric. Exp. Stn. Spec. Rep. 80. 11 pp.
6. Frosheiser, F. I. 1957. Studies on the etiology and epidemiology of *Diaporthe phaseolorum* var. *caulivora*, the cause of stem canker of soybeans. Phytopathology 47:87-94.
7. Hobbs, T. W., and Phillips, D. V. 1985. Identification of *Diaporthe* and *Phomopsis* isolates from soybean. (Abstr.) Phytopathology 75:500.
8. Keeling, B. L. 1982. A seedling test for resistance to soybean stem canker caused by *Diaporthe phaseolorum* var. *caulivora*. Phytopathology 72:807-809.
9. Krausz, J. P., and Fortnum, B. A. 1983. An epiphytotic of Diaporthe stem canker of soybean in South Carolina. Plant Dis. 67:1128-1129.
10. Morgan-Jones, G. 1985. The *Diaporthe/Phomopsis* complex on soybean: Morphology. Pages 1-7 in: Proceedings of the Conference on the *Diaporthe/Phomopsis* Disease Complex on Soybean. Agric. Res. Serv. U.S. Dep. Agric. 89 pp.
11. Morgan-Jones, G., and Backman, P. A. 1984. Characterization of southern biotypes of *Diaporthe phaseolorum* var. *caulivora*, the causal organism of soybean stem canker. (Abstr.) Phytopathology 74:815.
12. Phillips, D. V. 1984. A selective medium for *Diaporthe phaseolorum* var. *caulivora*. (Abstr.) Phytopathology 74:815.
13. Sciumbato, G. L., and Keeling, B. L. 1985. Effects of planting date and inoculation date on stem canker of soybeans. (Abstr.) South. Soybean Dis. Workers Proc. 12:69.
14. Smith, C. M., Davis, A. E., and Fielding, M. J. 1985. Environmental factors affecting development of Diaporthe stem canker in soybean seedlings. (Abstr.) South. Soybean Dis. Workers Proc. 12:81.