Effect of Temperature and Wetness Period on Recovery of the Southern Biotype of *Diaporthe phaseolorum* var. *caulivora* from Soybean

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ABSTRACT

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The effect of temperature and wetness period on the recovery of the southern biotype of *Diaporthe phaseolorum* var. *caulivora* was determined using 2-week-old soybean seedlings (cv. Walters) grown in the greenhouse and inoculated with 10^6 ascospores per ml of the fungus. Inoculated seedlings were placed in a dew chamber at either 15, 20, 22, 25, 27, 30, 32, or 35°C. Four randomly selected seedlings were removed after 8, 24, 36, 48, 72, and 96 h, air dried at room temperature, and stems and petioles of each plant were cut into 1-cm sections. Stem and petiole sections were surface disinfested and placed on potato-dextrose agar to determine incidence of recovery of the fungus. The fungus was recovered from a greater percentage of stem and petiole sections of plants incubated at 22 to 30°C than at 20 or 32°C. The lowest recovery was at 15 and 35°C. A multiple regression equation was developed that significantly (R^2 = 0.847, $P < 0.0001$) related temperature and wetness period to recovery. This equation predicted the optimal temperature for recovery to be 25.8°C. In vitro growth and germination of *D. phaseolorum* var. *caulivora* was fastest at 25 and 30°C, followed by 20 and 15°C, in that order. At 35°C, in vitro growth did not occur, but in vitro germination was faster than at 20°C but slower than at 25 or 30°C. In vitro growth was significantly correlated to recovery of the fungus at 72 ($R = 0.93$, $P = 0.02$) and 96 h ($R = 0.90$, $P = 0.04$), but germination was not related to recovery at any time sampled.

Stem canker, caused by the southern biotype of *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *caulivora* K. L. Athow & R. M. Caldwell, causes severe yield losses of soybean (*Glycine max* (L.) Merr.) in the southern United States (1,2). Characterized by distinct foliar and stem symptoms, stem canker occurs during reproductive development. These symptoms appear after a long latent period and result from infections during early vegetative growth (3). This long latent period has made it difficult to characterize the environmental conditions associated with infection.

Previous work has related duration of free moisture and temperature to infection of soybean by *D. phaseolorum* var. *caulivora*. Smith and Fielding (9) found that small lesions only formed when inoculated seedlings were exposed to long periods of leaf wetness and high humidity. Similarly, Damicone et al. (3) found cankers developed after 24 h of wetness in a greenhouse mist chamber. Highest levels of disease incidence and severity occurred after 48 and 96 h of wetness, respectively.

Temperature also plays an important role in infection. Ploetz and Shokes (6) exposed inoculated seedlings to 48 h of wetness at temperatures from 10 to 40°C and determined the percent recovery from petiole bases. They found that optimum recovery occurred at 28 and 34°C with low recovery levels at 10, 16, and 22°C and no recovery at 40°C. In another study, Keeling (5) reported that in vitro growth of two isolates of the southern biotype of *D. phaseolorum* var. *caulivora* was fastest at 25 to 30°C with little growth at 10 or 35°C.

The objectives of this research were to determine the relationship of temperature and wetness period duration to recovery of the pathogen from soybean and to determine the effect of temperature on in vitro growth and germination of the pathogen. A preliminary report has been published (8).

MATERIALS AND METHODS

Inoculum. A single-spore isolate of *D. phaseolorum* var. *caulivora* originating from a soybean with stem canker in southwestern Arkansas was used for all tests. Inoculum was produced in 9-cm plastic petri dishes containing potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI) with two to four pieces (3 to 4 cm long) of sterilized senescent soybean stem segments embedded in the medium. The stem sections were sterilized by soaking in deionized water for 30 min and autoclaving for 45 min on two successive days. A plug of mycelium was placed in the center of each plate. The plates were sealed (Parafilm, American National Can, Greenwich, CT) and incubated at room temperature (22°C) and light for 6 to 8 weeks. Ascospores were collected by flooding the plate with deionized water and rubbing the surface with a metal spatula. The resulting suspension was filtered through four layers of cheesecloth. The plates were reflooded and the procedure repeated. Inoculum concentration was adjusted to 10^6 ascospores per ml, and two drops per liter (approximately 1 ml) of Tween 20 was added as a surfactant.

Recovery. Procedures for assessing recovery from soybeans of *D. phaseolorum* var. *caulivora* were similar to those used in an earlier study of *Phomopsis longicolla* (7). Soybean plants (cv. Walters) were grown in 10 × 10 cm square plastic pots in potting mixture (Sunshine All-Purpose Mix, Fisons Horticulture, Belleuve, WA), one plant per pot, for 2 weeks in the greenhouse at temperatures from 22 to 30°C under natural light. The plants were sprayed with inoculum suspensions until run-off using a hand sprayer. The seedlings were then placed in a dew chamber at either 15, 20, 22, 25, 27, 30, 32, or 35°C. Four replicate plants were randomly removed after 8, 24, 36, 48, 72, and 96 h and placed under moving air at room temperature (22°C) to hasten drying. Plants were dry in 15 min. Incubation of plants at room temperature for up to 74 h after drying did not affect the level of recovery from stem and petiole sections (J. C. Rupe, unpublished data). The experiment was conducted three times at each temperature.

The last sample removed from the dew chamber was allowed to dry for 2 h and the stems and petioles were cut into 1-cm sections. There was an average of 20 stem and petiole sections per plant. The sections were surface disinfested by dipping in 95% ethanol and soaking in 0.5% NaOCl (Chlorox, The Chlorox Corp., Oakland, CA) for 5 min and then rinsing in sterile water. The plant pieces were placed on PDA acidified with 24 drops per liter of 10% lactic acid and containing 18 drops per liter of a nonionic surfactant (polygly-

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col ether, Tergitol NP10, Sigma, St. Louis, MO) in 9-cm plastic petri dishes. The dishes were incubated for 1 week at room temperature (22°C), and the proportion of stem and petiole pieces with *D. phaseolorum* var. *caulivora* was determined per plant. All treatments were assayed at the end of each experiment.

In vitro growth. The effect of temperature on in vitro growth on PDA of the same isolate used for the inoculation studies was determined. A 0.5-cm plug of mycelium removed from the edge of a growing colony was placed in the center of a 9-cm plastic petri dish containing PDA. The dishes were sealed with Parafilm and placed in incubators at either 15, 20, 25, 30 or 35°C. Colony diameter was measured at least three times in two perpendicular directions, and the rate of colony growth was determined using linear regression. There was one dish per replication, four replications per experiment, and three experiments per temperature.

In vitro germination. Inoculum was increased as described above except Tween 20 was not added. One milliliter of inoculum suspension was spread evenly over the surface of a 9-cm plastic petri dish containing 2% water agar (Moorehead Inc., San Diego, CA) acidified with 24 drops per liter of 10% lactic acid. Dishes were sealed in plastic bags and placed in an incubator at either 15, 20, 25, 30, or 35°C. Germination was determined periodically by cutting 1-cm^2 blocks of agar from the dish, placing the agar block on a slide, staining with lactophenol cotton blue, placing a cover slip over the block, and counting germinated and ungerminated ascospores. Germination was determined on a minimum of 100 spores per replication. A spore was considered germinated when the length of the germ tube was at least equal to the width of the spore. Germination was determined until at least 50% of the spores had germinated. The time to 50% germination was calculated by linear interpolation between the two times with germination levels nearest 50%. There was one dish per replication, four replications per experiment, and three experiments per temperature.

Analysis. Analysis of the recovery of *D. phaseolorum* var. *caulivora* data was by multiple regression. Initial analysis revealed that the multiple infection transformation [ln(1/(1 − y))] (4) was appropriate for all temperatures when recovery was determined separately at each temperature. The multiple infection transformation was selected to transform the data based on the biology of the system (4) and on comparison of the adjusted coefficients of determination, scatter plots, and residuals of the transformed and nontransformed data. In developing the multiple regression model, various combinations of temperature, wetness period and temperature by wetness period were evaluated. Parameters were excluded from the model that did not significantly contribute to the model or that did not greatly reduce the mean square error of the model. In vitro growth was analyzed as a randomized complete block with five temperatures and three replications. Experiments were treated as replications. Comparisons were made of growth rates calculated by linear regression at each temperature in each experiment. In vitro germination data from each of the three experiments were treated as a randomized complete block design with five temperatures and four replications. Where appropriate, means were compared by Duncan's multiple range test. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, NC).

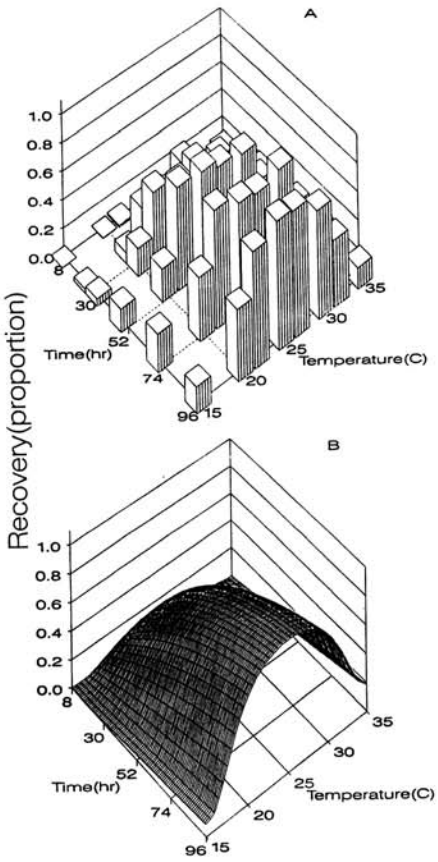

Fig. 1. Recovery of the southern biotype of *Diaporthe phaseolorum* var. *caulivora* from stem and petiole sections of artificially inoculated 2-week-old greenhouse grown soybeans (cv. Walters) with time at various temperatures: **(A)** actual results; or **(B)** predicted results from a multiple regression model.

RESULTS

Recovery. Little or no recovery occurred after an 8-h wet period at any temperature, but the percentage of stem and petiole sections yielding *D. phaseolorum* var. *caulivora* rapidly increased with longer wet periods at temperatures from 22 to 30°C (Fig. 1A). Maximum recovery of 77 to 88% was reached after 72 to 96 h. For a given wet period, the percent recovery was greatest at 22 to 30°C, less at 20 and 32°C, and least at 15 and 35°C.

A model was developed relating temperature and dew period to recovery (proportion of stem and petiole pieces with the fungus) transformed by the multiple infection transformation (4) (Table 1). This model significantly fit the data (R^2 = 0.847, $P < 0.0001$) and predicted the optimum temperature for recovery to be

Table 1. Model describing the effect of temperature and dew period on recovery of the southern biotype of *Diaporthe phaseolorum* var. *caulivora* from artificially inoculated soybean seedlings[w]

Variable[x]	Parameter[y]	Standard error
Intercept	−0.023643	0.03744945
Time	0.933049	0.14054896
Temp × Time	−0.167366	0.02438243
Temp^2 × Time	0.010769	0.00153409
Temp^3 × Time	-2.9231×10^4	0.00004164
Temp^4 × Time	2.8441×10^6	0.00000041
$R^2 = 0.847$[z]		

[w] Recovery of *D. phaseolorum* var. *caulivora* from artificially inoculated soybean seedlings expressed as the multiple infection transformation (ln(1/(1 − Y)) (4) of the proportion of 1-cm stem and petiole sections with the fungus. Seedlings (cv. Walters) were 2 weeks old when inoculated with 10^6 ascospore per ml of the fungus.

[x] Time valid for 8 to 96 h, temperatures for 15 to 35°C.

[y] All parameters significant at the $P < 0.0001$ level except the intercept, which was not significant.

[z] Overall regression significant at $P < 0.0001$.

Table 2. Comparison of the rate of in vitro growth and germination of the southern biotype of *Diaporthe phaseolorum* var. *caulivora* at various temperatures[w]

Temperature (C)	Growth rate (mm/h)[x]	Hours to 50% germination[y]
15	0.88 c[z]	35.5 d
20	1.2 b	16.9 c
25	1.6 a	9.7 a
30	1.6 a	9.2 a
35	0.0 d	13.6 b

[w] Growth study conducted on potato-dextrose agar. Germination conducted on water agar acidified with 24 drops per liter of 10% lactic acid.

[x] Growth rates based on linear regression of a minimum of three measurements per replication. There were four replications per experiment and three experiments with five temperatures. All regression equations were significant ($P < 0.001$).

[y] Germination was determined periodically until at least 50% of the ascospores had germinated. Hours to 50% germination was determined by interpolation between the two samples closest to 50% germination. A spore was considered germinated if the germ tube was at least as long as the width of the spore. There were four replications per experiment and three experiments with five temperatures.

[z] Means within a column followed by different letters were significantly different at $P = 0.05$ by Duncan's multiple range test.

25.8°C. The model predicted a broad range of near optimal temperatures from the lower 20s to 30°C with a rapid reduction in recovery at higher and lower temperatures (Fig. 1B). In this optimal range, the model predicted rapid increases in recovery in the first 48 h and slower increases thereafter.

In vitro growth. In vitro growth of *D. phaseolorum* var. *caulivora* was fastest at 25 and 30°C, followed by 20 and 15°C (Table 2). No growth occurred at 35°C. Growth rates were highly correlated to recovery at 72 h ($R = 0.93$, $P = 0.02$) and 96 h ($R = 0.90$, $P = 0.04$).

In vitro germination. Germination was fastest at 25 and 30°C, followed by 20 and 35°C, and was slowest at 15°C (Table 2). Germination was not significantly correlated to recovery at any assessment time.

DISCUSSION

Recovery is used in this paper as an estimation of infection. It is assumed that, because the plants were dried and surface disinfested, recovery of *D. phaseolorum* var. *caulivora* from the plant resulted from infection and not surface contamination. No recovery of the fungus occurred without a wet period using the assay methods described in this paper (J. C. Rupe, unpublished data). It is not known how many infections are necessary for recovery of the fungus from a plant section, but it is assumed that increased recovery is due to increased infection.

Recovery of *D. phaseolorum* var. *caulivora* from soybean seedlings was highly dependent on temperature and wetness period. At most temperatures, recovery began between 8 and 24 h but did not reach a maximum until 72 to 96 h after inoculation (Fig. 1A). These data were fit well by a multiple regression model ($R^2 = 0.847$) that predicted a broad range of near-optimal temperatures and long dew periods for high levels of recovery (Table 1, Fig. 1B). This need for long periods of wetness for maximum recovery agrees with the report by Damicone et al. (3) that maximum incidence and severity occurred after 48 and 96 h of wetness, respectively. In the study presented here, recovery increased in a similar manner at temperatures from 22 to 30°C, but slowed dramatically outside of this range (Fig. 1A,B). This sharp drop in recovery above 30 and below 22°C and the long dew period required for recovery in the optimum temperature range suggests that it is unlikely that infection will occur at temperatures outside the 22 to 30°C range. These findings differ somewhat from those of Ploetz and Shokes (6) who found high levels of recovery at 28 and 34°C, low levels at 10 to 22°C, and no recovery at 40°C. While the temperature optimum of 34°C was higher than that reported in the present study, the study by Ploetz and Shokes does confirm that the rate of recovery drops off sharply outside the optimum temperature range. Differences in results may be due to their single sample at 48 h and assaying only the petiole bases instead of all of the stems and petioles, or it may reflect regional differences in the pathogen.

If there are regional differences among strains of *D. phaseolorum* var. *caulivora* in response to temperature, quantifying these differences would be a critical factor in understanding the epidemiology of stem canker. One way of determining these differences might be to compare the in vitro growth responses of different strains across a range of temperatures, since in vitro growth and recovery were highly correlated. This high correlation also implies that establishment of the fungus in the host tissue is more critical to infection than is germination.

The study reported here reveals that infection of soybeans by *D. phaseolorum* var. *caulivora* (as estimated by recovery) requires a long wet period with warm temperatures. Since this is a splash-dispersed pathogen, infection is likely to occur after a rain that is followed by dew periods of at least 24 h with temperatures between 22 and 30°C. Outside this temperature range, infection is unlikely. This partially explains why delayed planting often results in less stem canker (1). In Arkansas, the weather becomes drier and warmer as the season progresses so that rain events are usually not followed by an extended wetness period. Lack of stem canker in early plantings (early to mid April), which often experience long wet periods following a rain, may be due to temperatures too low to permit infection. Research is continuing that will incorporate the model developed in this paper into a model for scheduling early season applications of systemic fungicides to control stem canker.

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LITERATURE CITED

1. Backman, P. A., McGee, D. C., and Morgan-Jones, G. 1989. Stem canker. Pages 41-43 in: Compendium of Soybean Diseases. 3rd ed. J. B. Sinclair and P. A. Backman, eds. American Phytopathological Society, St. Paul, MN.
2. Backman, P. A., Weaver, D. B., and Morgan-Jones, G. 1985. Soybean stem canker: An emerging disease problem. Plant Dis. 69:641-647.
3. Damicone, J. P., Berggren, G. T., and Snow, J. P. 1987. Effect of free moisture on soybean stem canker development. Phytopathology 77: 1568-1572.
4. Gregory, P. H. 1948. The multiple infection transformation. Ann. Appl. Biol. 35:412-417.
5. Keeling, B. L. 1988. Influence of temperature on growth and pathogenicity of geographic isolates of *Diaporthe phaseolorum* var. *caulivora*. Plant Dis. 72:220-222.
6. Ploetz, R. C., and Shokes, F. M. 1987. Factors influencing infection of soybean seedlings by southern *Diaporthe phaseolorum*. Phytopathology 77:786-790.
7. Rupe, J. C. 1990. Effect of temperature on the rate of infection of soybean seedlings by *Phomopsis longicolla*. Can. J. Plant Pathol. 12:43-47.
8. Rupe, J. C., and Sutton, E. A. 1994. Effect of temperature on infection of soybeans by the southern biotype of the stem canker pathogen, *Diaporthe phaseolorum* var. *caulivora*. (Abstr.) Phytopathology 84:1120.
9. Smith, C. M., and Fielding, M. J. 1985. Environmental factors affecting development of *Diaporthe* stem canker in soybean seedlings. (Abstr.) Page 81 in: Proc. Ann. Meet. Southern Soybean Dis. Workers, 12th. 12:81.