Effect of Free Moisture on Soybean Stem Canker Development

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ABSTRACT

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The influence of duration and type of free moisture on soybean stem canker development was studied by inoculating plants of breeding line J77-339 with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora* and maintaining free moisture on the plants in mist chambers in the greenhouse. Wetting periods of 0–144 hr preceded exposure of plants to either dry or discontinuous wetting (8 hr/day) treatments for the remainder of the experiment. An additional treatment consisted of continuous wetting for the entire experiment. The pathogen was isolated from symptomless plants 7 days after inoculation in all treatments except one not receiving moisture. Isolation frequency declined for all treatments 30 days after inoculation. More than 24 hr of free moisture preceding dry treatment was required for stem canker development. Increases in incidences of stem canker and dead plants with wetting duration were sigmoidal for the dry treatment. Increase in canker number per plant for the dry treatment was quadratic. The length of the incubation period declined linearly with wetting duration from 48 to 144 hr preceding both dry and discontinuous moisture treatments. Maximum levels of stem canker incidence and severity and minimum incubation periods were achieved both with discontinuous wetting after 48- and 96-hr wetting periods and with continuous wetting for the entire experiment. Stem canker incidence, canker number, and incubation period, but not incidence of dead plants, for dry treatments approached levels achieved with discontinuous wetting treatments at 144 hr wetting. Moisture requirements for stem canker development were not rigid. Disease incidence and severity and length of the incubation period were related more to the total moisture duration than to the type (continuous vs. discontinuous).

Stem canker of soybean (*Glycine max* (L.) Merr.), caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell, was an important disease in the midwestern United States and Canada during the late 1940s to the early 1950s (1,5,22). Stem canker is now a serious disease over much of the southern United States, with yield losses approaching 80% in severely affected fields planted with susceptible cultivars (6,8,21).

Ascospores and alpha-conidia of the pathogen are released in a gelatinous matrix from perithecia and pycnidia, respectively, formed on stem debris (2,12). Spore deposition on plants presumably occurs via splashing rain. When applied as an aqueous suspension to plants in the vegetative stage, ascospores and alpha-conidia of the pathogen incited stem canker symptoms after an incubation period of 50–75 days (12). Incubation of inoculated plants under free moisture conditions is required for stem canker development, although the type and duration is not well defined (7,12,14,18). We report here on the relationship of continuous and discontinuous free moisture to stem canker incidence, severity, and length of the incubation period.

MATERIALS AND METHODS

A single ascospore isolate of *D. p.* var. *caulivora* was obtained from a cankered soybean plant collected in St. Landry Parish, LA. Ascospores and conidia of the isolate were stored on silica gel at 4 C (20). The isolate was grown in plastic petri dishes containing air-dried, double-autoclaved soybean stem pieces (13) on the surface of potato-carrot agar acidified to pH 4.5 with lactic acid (20). Ascospores and alpha-conidia were produced on the stem pieces after 21–28 days of incubation at 21–23 C. Ascospores and conidia were removed by flooding the surface of the dishes with sterile distilled water and gently scraping the surface of the agar and stem pieces with a rubber policeman. The suspensions from several dishes were pooled and filtered through six layers of cheesecloth. The spore concentration was determined with a hemacytometer, adjusted to approximately 1×10^6 spores per milliliter, and then used immediately.

The soybean breeding line J77-339, highly susceptible to stem canker (7), was used. Autoclaved clay pots (21-cm diameter) were filled with a potting mix containing peat, perlite, and methyl bromide-fumigated silt loam soil (2:1:3, v/v). Pots were sown with seed treated with soybean inoculant (Legume Aid, Kalo Inc., Overland Park, KS) and maintained in the greenhouse at 22–30 C. Plants were thinned to three or four per pot. Slow-release fertilizer (14:14:14, % N:P:K) (Osmocote, Sierra Chemical Co., Milpitas, CA) was added to each pot (8 g per pot) at the three-leaf (V_3) growth stage (4). Plants were grown under 14-hr daily supplemental fluorescent lighting (F40/CW bulbs, Sylvania Co.) to retard flowering. Plants at the five-leaf (V_5) growth stage (about 35 days after planting) were inoculated by spraying entire plants to runoff with the spore suspensions, using a compressed air sprayer (model 153, R. E. Chapin Manufacturing Works, Inc., Batavia, NY).

Free moisture was applied to plants using a mist system suspended over open-top polyethylene chambers ($1.22 \times 1.22 \times 1.90$ m) constructed on greenhouse benches. Eight hollow cone mist nozzles (model SF-2, Spraying Systems Co., Wheaton, IL) were suspended at an equidistance over chambers. The flow of

deionized water through the nozzles was controlled with solenoid valve switches and cycle timers. Free moisture was maintained by applying mist 15 sec every 10 min. Supplemental lighting (three 40W fluorescent bulbs per chamber) for 14 hr/day was added until the 10-leaf (V_{10}) growth stage. Plants receiving the dry treatment were placed in chambers identical to the mist chambers except that no mist was applied. Plants in the dry chambers were carefully watered as needed thereafter to avoid splashing.

The experiment consisted of a completely randomized design with 12 treatments and four replicates and was done twice. A replicate consisted of a pot containing three plants. In addition, one pot with four plants was included in each treatment for the purpose of isolating the pathogen. Plants were inoculated at 1600 hours and placed in the mist chambers. Five randomly selected pots were removed from continuous free moisture treatment after intervals of 0, 24, 48, 72, 96, 120, and 144 hr and placed in the dry chambers for the remainder of the experiment. Five pots were also removed at random after 0, 48, 96, and 144 hr and placed in chambers for discontinuous wetting. Discontinuous wetting treatment consisted of 8-hr wetting periods per day (0000 to 0800 hours) for the remainder of the experiment. One treatment consisted of plants receiving continuous free moisture for the entire experiment.

Isolations were made 7 and 30 days after inoculation, using a medium selective for *D. p.* var. *caulivora* (11). Two plants per treatment were sampled on each isolation date in both experiments. Three 1-cm-long sections of the stem from the third and fifth nodes per plant were made. The sections were surface-sterilized for 5 min in 0.5% aqueous NaOCl, blotted dry, and placed on the surface of the selective medium contained in plastic petri dishes. The plates were sealed with Parafilm and incubated for 10 days on a laboratory bench at 21–23 C. The frequency of pathogen recovery from the 24 stem sections per treatment was determined and expressed as percent.

The length of the incubation period was measured as days after inoculation to canker appearance and was determined by sequential evaluations. Evaluation dates were 61, 83, and 106 days after inoculation in experiment one and 56, 72, and 98 days in experiment two. The sampling dates corresponded to full pod (R_4), beginning seed (R_5), and full seed (R_6) stages of development. Regression analysis of incubation period on wetting period for both dry and discontinuous wetting treatments was performed. Observations were single symptomatic plants. Homogeneity of the slopes and levels of the predicted lines was tested by analysis of covariance (10).

Disease incidence and severity were evaluated at the R_6 stage. Stem canker incidence was measured as the proportion of plants with cankers. Disease severity measurements consisted of the proportion of dead plants, number of cankers per plant, and canker length (cm). Regression analysis of disease incidence or severity variables on wetting period for both dry and discontinuous wetting treatments was performed. Observations for incidences of diseased and dead plants and canker number per plant were replicate (three plants per pot) means. Observations for canker length were single symptomatic plants. Criteria of model aptness were $P>F$ for regression (<0.05), $P>F$ for lack of fit (>0.05), and the coefficient of determination (R^2)(10).

RESULTS

D. p. var. *caulivora* was recovered 7 days after inoculation from stem sections representing all treatments except the one receiving no free moisture (Table 1). Pathogen recovery ranged from 4% for 24- and 48-hr wetting periods preceding dry treatment to 38% for plants receiving continuous wetting the entire experiment. No symptoms (lesions or cankers) were apparent on any of the plants sampled at 7 days. Pathogen recovery declined for all treatments 30 days after inoculation (Table 1). Percent isolation ranged from 0 to 25, with the highest values from plants receiving discontinuous moisture after 48- and 96-hr wetting periods. The pathogen was isolated 30 days after inoculation from symptomless stem sections and from those with 1- to 3-mm-long elliptical, reddish brown lesions.

Stem canker did not develop on plants receiving 0- or 24-hr free moisture preceding dry treatment (Fig. 1). Increase in stem canker incidence for wetting periods from 48 to 144 hr was sigmoidal. The integrated (sigmoid) form of the Gompertz growth model (3) adequately described increase in stem canker incidence (y) with wetting duration preceding dry treatment (Fig. 1). The fitted model was $y = 0.75*\exp(-13.68)*\exp(-0.03)*x$ ($R^2 = 0.79$, $P>F$ for lack of fit = 0.55). In comparison to dry treatment, discontinuous wetting increased stem canker incidence after 0-, 48-, and 96-hr, but not 144-hr, wetting (Fig. 1). Maximum stem canker incidence was at 48- and 96-hr wetting. The relationship between stem canker incidence (y) and wetting duration for discontinuous wetting

TABLE 1. Influence of type and duration of free moisture treatment on recovery of *Diaporthe phaseolorum* var. *caulivora* from J77-339 soybean stem pieces

Free moisture duration (hr)	Treatment[a]	Percent isolation[b] after inoculation	
		7 days	30 days
0	Dry	0	0
	Discontinuous	12	0
24	Dry	4	0
48	Dry	4	0
	Discontinuous	33	25
72	Dry	12	4
96	Dry	25	4
	Discontinuous	38	12
120	Dry	17	8
144	Dry	21	4
	Discontinuous	25	4
Continuous[c]	...	38	4

[a] Dry = no free moisture after wetting interval; discontinuous = 8 hr/day free moisture after wetting interval for duration of experiment.
[b] Percent isolation of *D. p.* var. *caulivora* from 24 stem sections per treatment.
[c] Free moisture throughout experiment.

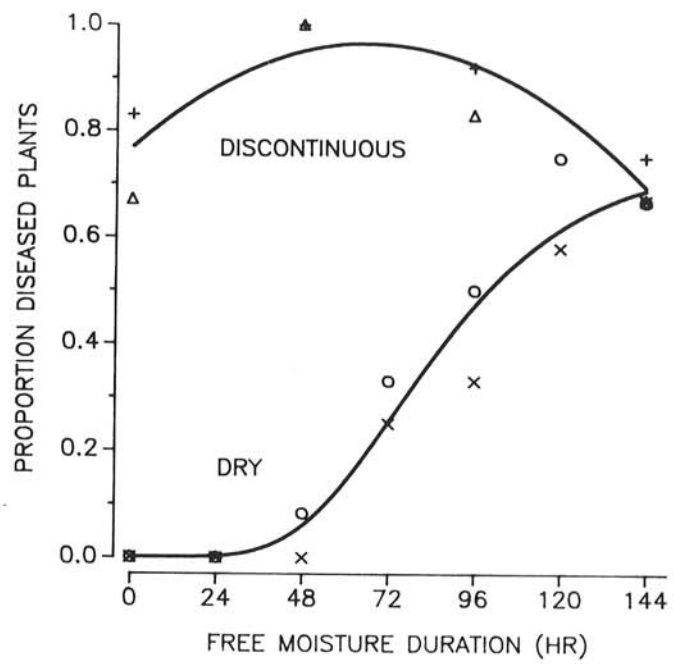

Fig. 1. Relationship of stem canker incidence to wetting duration after inoculation (0–144 hr) preceding dry (no free moisture) or discontinuous wetting (8 hr/day free moisture) treatments. J77-339 soybean plants were inoculated with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora*. Lines depict models fit to eight replicate subsamples. Data points represent mean values of 12 plants (x = dry, experiment 1; o = dry, experiment 2; Δ = discontinuous, experiment 1; + = discontinuous, experiment 2).

treatment was quadratic, $y = 0.76 + 0.006x - 0.0004x^2$ ($R^2 = 0.33$, $P>F$ for lack of fit = 0.19).

The number of cankers per plant (y) increased with wetting duration for the dry treatment (Fig. 2). The increase was quadratic and given by $y = -0.02 - 0.001x + 0.00007x^2$ ($R^2 = 0.71$, $P>F$ for lack of fit = 0.64). Canker number was greater following 0-, 48-, and 96-hr, but not 144-hr, wetting for discontinuous wetting treatment in comparison to dry treatment (Fig. 2). Maximum canker number occurred with discontinuous wetting treatment at 48, 96, and 144 hr. There was no significant relationship between canker number (y) and wetting duration for the discontinuous wetting treatment.

Increase in incidence of dead plants (y) with wetting duration for dry treatment was sigmoidal (Fig. 3). The fitted Gompertz model was $y = 0.30*\exp(-361.5)*\exp(-0.07)*x$ ($R^2 = 0.51$, $P>F$ for lack of fit = 0.93). The incidence of dead plants was greater at all wetting periods for discontinuous wetting treatment in comparison to dry treatment (Fig. 3). The greatest incidence of dead plants was with discontinuous wetting following 96 hr. No significant relationship was found between dead plants (y) and wetting duration for the discontinuous wetting treatment.

Canker length was not related to moisture duration for dry or discontinuous moisture treatments. Mean length of cankers for symptomatic plants receiving dry treatment ranged from 5.3 to 16.0 cm (Table 2). Similarly, mean length of cankers for discontinuous wetting treatment ranged from 8.7 to 17.9 cm (Table 2).

The length of the incubation period (y) declined linearly with moisture duration from 48- to 144-hr wetting for the dry treatment (Fig. 4A). Predicted incubation periods (y) for symptomatic plants receiving dry treatment were given by $y = 120.9 - 0.4x$ ($R^2 = 0.45$, $P>F$ for lack of fit = 0.35). Incubation periods for discontinuous wetting treatment (y) were also linearly related to wetting duration, although considerable variation was evident (Fig. 4B). Predicted incubation periods for symptomatic plants receiving discontinuous wetting were given by $y = 72.8 - 0.07x$ ($R^2 = 0.10$, $P>F$ for lack of fit = 0.97). Slopes and levels of the regression equations were significantly different ($P<0.01$) according to analysis of covariance.

Disease incidence and severity and the length of the incubation period values for plants receiving continuous mist for the duration of the experiment were similar to maximum levels achieved with discontinuous moisture treatment. Mean values of stem canker incidence, canker number, incidence of dead plants, and length of the incubation period were 0.92, 2.5 per plant, 0.62, and 59.8 days, respectively.

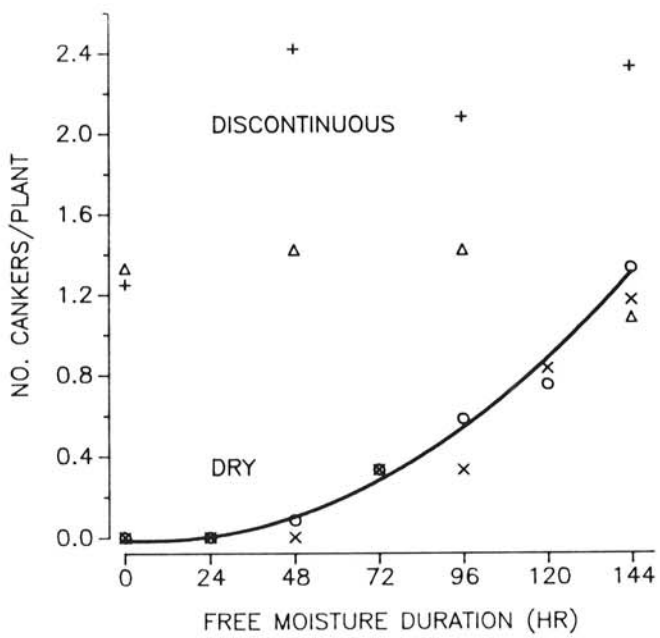

Fig. 2. Relationship of number of cankers per plant to wetting duration after inoculation (0–144 hr) preceding dry (no free moisture) or discontinuous wetting (8 hr/day free moisture) treatments. J77-339 soybean plants were inoculated with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora*. Line depicts model fit to eight replicate subsamples. Data points represent mean values of 12 plants (x = dry, experiment 1; o = dry, experiment 2; Δ = discontinuous, experiment 1; + = discontinuous, experiment 2).

DISCUSSION

Free moisture greatly affected stem canker incidence and development in highly susceptible J77-339 soybeans in the

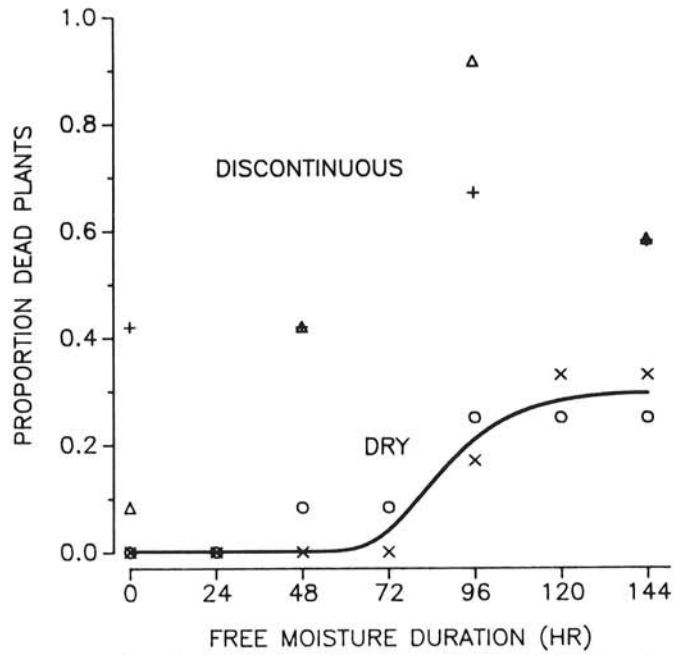

Fig. 3. Relationship of incidence of dead plants to wetting duration after inoculation (0–144 hr) preceding dry (no free moisture) or discontinuous wetting (8 hr/day free moisture) treatments. J77-339 soybean plants were inoculated with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora*. Line depicts model fit to eight replicate subsamples. Data points represent mean values of 12 plants (x = dry, experiment 1; o = dry, experiment 2; Δ = discontinuous, experiment 1; + = discontinuous, experiment 2).

TABLE 2. Effect of type and duration of free moisture on canker length on J77-339 soybeans inoculated with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora*

Free moisture duration (hr)	Treatment[a]	Cankers		
		Length (cm)	Number of symptomatic plants	Standard error
0	Dry	...	...	...
	Discontinuous	8.7	18	1.67
24	Dry	...	...	...
	Discontinuous	...	...	...
48	Dry	13.0	1	...
	Discontinuous	12.0	24	1.98
72	Dry	5.3	7	2.43
	Discontinuous	...	...	...
96	Dry	16.0	10	3.40
	Discontinuous	17.9	21	1.46
120	Dry	10.4	16	2.12
	Discontinuous	...	...	...
144	Dry	11.6	16	1.89
	Discontinuous	13.4	17	1.69

[a] Dry = no free moisture after wetting interval; discontinuous = 8 hr/day free moisture after wetting interval for duration of experiment.

greenhouse. Dry treatment after wetting intervals of 0–144 hr gave the minimum moisture requirements for disease development. Prolonged wetting of greater than 96 hr was required for >50% disease incidence for the dry treatment. Prolonged initial wetting was not required for >50% disease incidence preceding discontinuous wetting treatment (8 hr/day). Continuous wetting for the entire experiment gave the maximum levels of disease incidence and severity. Discontinuous wetting treatment resulted in disease incidence and severity values at or near the maximum levels. These results suggest that the moisture requirements for stem canker after inoculation are not rigid with respect to the type of moisture application.

Seasonal variation in stem canker outbreaks has been reported (2,14,16). This variation has been attributed in part to yearly differences in environmental conditions (2,14,16). Plant infection by ascospores and conidia of *D. p.* var. *caulivora* reportedly occurs at temperatures ranging from 10 to 34 C (14,18). Although this temperature range is rarely exceeded during rainy periods when soybeans are grown in Louisiana (Damicone, *unpublished*), high temperature could possibly limit infection after spore deposition.

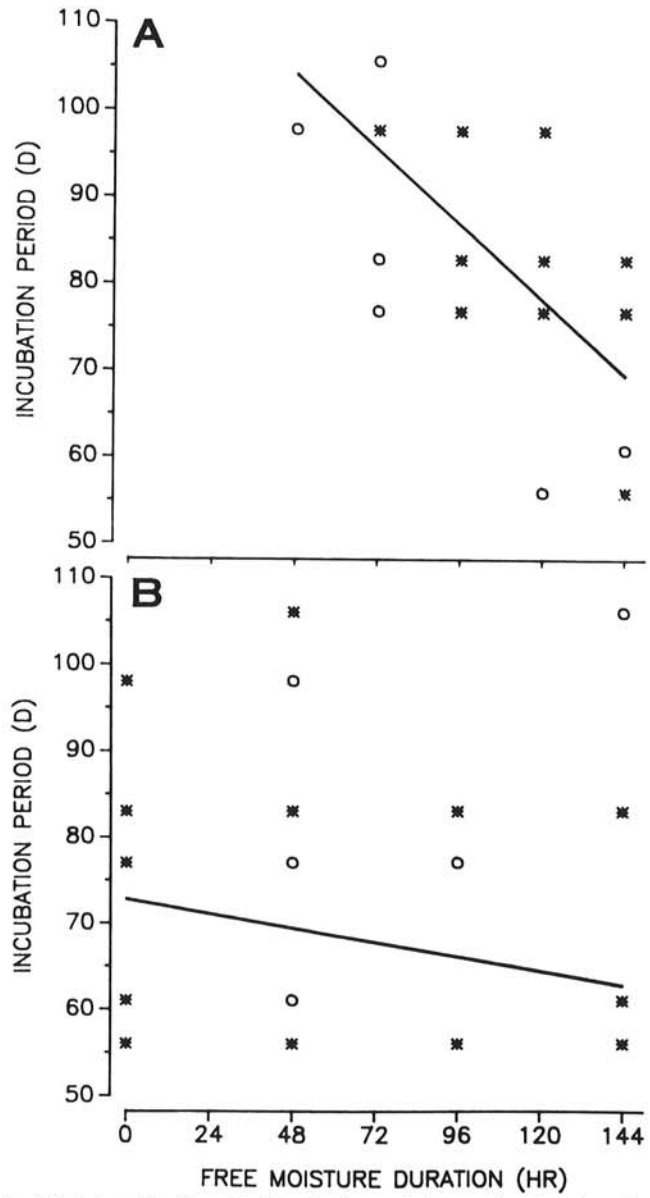

Fig. 4. Relationship of length of incubation period to wetting duration after inoculation (0–144 hr) preceding **A,** dry (no free moisture) or **B,** discontinuous wetting (8 hr/day free moisture) treatments. J77-339 soybean plants were inoculated with ascospores and conidia of *Diaporthe phaseolorum* var. *caulivora*. Lines depict models fit to single symptomatic plants. Data points: o = one plant, * = three or more overlapping points.

Late spore deposition in relation to the soybean stage of development can also reduce stem canker incidence in a given year (2,19). In addition, the level of cultivar susceptibility influences stem canker outbreaks (2,6,15,21). Results from this study and from others (7,12,18) indicate free moisture after spore deposition is an important factor regulating stem canker development.

The duration of free moisture affected stem canker severity as well as incidence. Increase in disease incidence and incidence of dead plants for the dry treatment resembled a sigmoid growth curve, with a plateau at 120- and 144-hr wetting periods. Berger (3) proposed the use of the linearized Gompertz growth model to characterize disease increase over time. Our use of a growth model involved the relationship of disease increase with wetting duration. This is similar to MacKenzie's (9) proposed use of arbitrary units of severity based on environmental factors favorable for disease development as a unit of epidemiologic time. Canker number per plant for the dry treatment did not yet reach a plateau at 120 and 144 hr. Levels of disease incidence and canker number for dry treated plants at the 144-hr wetting period approached levels achieved with discontinuous wetting treatment. Incidence of dead plants in the dry treatment, however, did not approach levels achieved by discontinuous wetting. This was attributed to the generally longer incubation periods for dry treatment compared with discontinuous wetting treatment. Wetting periods greater than 144 hr preceding dry treatment may result in disease incidence and severity values equal to those of discontinuous wetting treatment.

Canker length was not affected by the type or duration of free moisture. Canker length is reported to be a function of cultivar resistance (7) and is also related to position on plants and to insect feeding (17). In addition, the amount of healthy stem tissue (noncankered) available on a given stem influences canker length.

The length of the incubation period was influenced by the duration of free moisture. The observed decrease in incubation period with wetting duration preceding dry treatment and the further decrease with discontinuous wetting have important epidemiologic implications. Yield loss due to stem canker occurs when plants are killed before complete pod fill (2). Any factor contributing to plant death before this critical stage will enhance yield loss. The occurrence of spore deposition at late stages of soybean development also contributes to plant survival through this critical period (2,19).

Survival of *D. p.* var. *caulivora* on symptomless soybean plants is well documented (2,12,14,15). The pathogen was isolated from symptomless plants in this study 7 days after inoculation in treatments receiving as little as 24 hr of free moisture (Table 1). Apparently, the pathogen is able to survive on the host for this time period. Others have reported pathogen survival with as little as 12 hr of free moisture (12). Pathogen recovery in this study declined to low or undetectable levels after 30 days, suggesting that not all the pathogen "survival units" become established and cause stem canker. The mechanism of survival on or infection of symptomless plants is unknown.

This study demonstrated that once spore deposition has taken place, either a prolonged and continuous wetting event or many discontinuous wetting periods facilitate stem canker development. These results indicate stem canker development is related more to the total moisture applied and less to the type (continuous vs. discontinuous). Plants in the field are likely to be exposed to multiple wetting events of variable duration. Cumulative moisture duration and/or rainfall during the vegetative stages of growth may be useful for identification of high stem canker risk periods. Information about effects of free moisture and other factors such as temperature on field epidemics is needed. The identification of moisture as a key factor is important for focusing future research on the ecology and epidemiology of this disease.

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