

Influence of Soil Moisture on Formation of Perithecia and Pycnidia and Spore Release in *Diaporthe phaseolorum* var. *caulivora*

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ABSTRACT

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The formation of perithecia and pycnidia and the release of ascospores and conidia, respectively, by *Diaporthe phaseolorum* var. *caulivora* was determined on soil at matric potentials (ψ_m) between 0 and -0.32 MPa. *D. p. caulivora* infected internodes of susceptible soybean cv. Bedford were incubated at 24 ± 1 C on the surface of soil samples adjusted to $\psi_m = 0.0, -0.01, -0.02, -0.04, -0.08, -0.16, \text{ or } -0.32$ MPa. The number of perithecia and pycnidia formed and the number of sporulated perithecia and pycnidia that released ascospores and conidia, respectively, per square centimeter of soybean internode were counted at weekly intervals. Large numbers of perithecia ($24\text{--}93/\text{cm}^2$) formed at ψ_m between 0 and -0.08 MPa after incubation for 7 days, whereas fewer perithecia ($3\text{--}37/\text{cm}^2$) formed at -0.16 and -0.32 MPa after incubation for 21 days. Maximum

production of perithecia occurred at 14 days of incubation at 0 to -0.02 MPa, at 28 days at -0.04 MPa, and at 35 days at -0.08 MPa. The highest number of sporulated perithecia was produced at -0.04 MPa after incubation for 28 days. Ascospores and conidia were released from the perithecia and pycnidia, respectively, in a gelatinous matrix. The gelatinous matrix dried on the surface of perithecia after 7 days. Ascospores in the dried gelatinous matrix remained viable for 35 days. The basal internode produced the highest number of perithecia and sporulated perithecia. The number of pycnidia formed and the release of conidia were erratic at different matric potentials. These results suggest that factors other than soil moisture govern the production of pycnidia.

Additional keywords: epidemiology, *Glycine max*, soilborne, stem canker.

Stem canker of soybean (*Glycine max* (L.) Merr.) caused by *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *caulivora* K. L. Athow & R. M. Caldwell was an important disease in the late 1940s and early 1950s in the midwestern United States (1) and Canada (10). The disease ceased to be a problem in this area because of the use of resistant cultivars (2). Stem canker has become a serious problem in much of the southeastern United States in the last 15 yr (2,11,14,21,23,25,28,29). Yield losses as high as 80% have been reported when susceptible cultivars were planted (9,14,32). In 1983, stem canker resulted in an estimated \$37 million loss in the southeastern United States (2).

Typical stem canker disease cycles begin with the appearance of perithecia and pycnidia on overwintered stem debris (2). Ascospores and conidia are released from perithecia and pycnidia, respectively, in a gelatinous matrix (2,18) and are dispersed by splashing raindrops and windborne rain (2). Both ascospores and conidia are capable of inciting stem canker (18). Splash-dispersed ascospores and conidia land on petioles, petiole bases, and stem tissue of seedlings (5,19), and subsequently cause infection. Symptoms appear on the infected plants during the reproductive growth stages after 50–75 days of incubation (5,21). The cankers elongate, girdle the stems, and eventually kill the plants (5,21). Some infected plants may remain symptomless throughout the growing season (2,18,20,21). The mechanisms of infection and survival in symptomless plants are unknown. Final disease severity is closely related to incidence of stem canker during early vegetative growth stages (2,24,28,32), and the potential exists to use initial stem canker incidence to predict final disease severity. However, no predictive models have yet been developed.

Cultivar susceptibility (2,6,9,11,12,32), temperature (13,20), free

moisture duration (5,18), and inoculum density (20) influence host infection. Inconsistencies in stem canker development from year to year in a given area may be attributable to variations in one or more of these factors (2). Soybean cultivars vary greatly in susceptibility to stem canker (9,11,32), and use of resistant cultivars has reduced disease incidence and severity (2). Infections by ascospores and conidia occur over a wide temperature range (20), and free moisture is required for both infection and subsequent canker development (5,18). Incubation period and disease incidence and severity are affected more by total moisture duration than by the frequency of moisture availability (5). Spread of stem canker from inoculum point sources is related to cultivar susceptibility and cumulative rainfall (6). Spread of the disease generally occurs in the direction of water movement (6).

Although seed has been suspected as a source of primary inoculum for *D. p. caulivora* infections (15), this has not been confirmed (2). Overwintered stem debris provides much of the primary inoculum for stem canker infection (2), and cultural practices directed towards reducing or avoiding this inoculum, such as increased tillage (23), crop rotation (2,24), and delayed planting (2), are recommended to reduce infection. The free moisture requirements for post-inoculation stem canker progress have been studied (5,18); however, the role of the soil environment in the production of primary inoculum is uncertain. In a recent study (22), increased stem canker infections occurred on soybeans grown in soils with high organic matter, pH, and soil moisture, but low potassium.

The influence of soil moisture on the formation of perithecia and pycnidia and on the subsequent release of ascospores and conidia, respectively, is directly related to the amount of effective primary inoculum. The role of soil moisture in sporulation by *D. p. caulivora* is, therefore, important in the epidemiology of the disease. The objective of this study was to determine the effects of soil moisture on the formation of perithecia and pycnidia and

on spore production on *D. p. caulivora* infected internodes. Portions of this work have been reported (31).

MATERIALS AND METHODS

Plant material used in this study was collected from the Ben Hur Research Station at Baton Rouge, LA, during the 1989 soybean-growing season. Soybean plants of a susceptible cultivar, Bedford, each with 40% stem canker severity (estimated on a 0–100% scale on the main stem at R5 stage [8]), were tagged. At maturity, these plants were severed at the soil level. The harvested plants were transported to a greenhouse and air dried on benches for 15 days.

Soil and treatments. Mississippi River alluvial soil (very fine sandy loam), pH 6.6 and 0.34% organic matter, was collected from the Ben Hur Research Station in December 1989. The soil was screened through a 2-mm-mesh sieve and air dried in the greenhouse.

Portions (200 g) of air-dried soil were equilibrated at 0.0, -0.01, -0.02, -0.04, -0.08, -0.16, or -0.32 MPa with 0.1-, 0.3-, or 1.5-MPa ceramic plates in an N₂-pressurized soil water extractor (Soil Moisture Equipment Co., Santa Barbara, CA) at room temperature. The equilibrated soil samples were placed in clear plastic storage boxes (12 × 6.5 × 5.5 cm) with four and three replicates for each ψ_m treatment in experiments 1 and 2, respectively. The lids were closed immediately to prevent moisture loss or gain. Percentage of moisture determinations for each initial ψ_m were made gravimetrically (3), and a soil moisture release curve was developed.

Differences in the number of perithecia and pycnidia produced on three lower internodes of an infected soybean plant were determined. The internodes were soaked in sterile, distilled water for 2 min and blotted dry to simulate the conditions in the field, where wetting of the soil and soybean debris occurs simultaneously. The three internodes from a particular plant were put into a box, and this represented a replicate of a moisture treatment. The internodes were placed on the surface of the soil, and the box was sealed with Parafilm M (American Can Company, Dixie/Marathon, Greenwich, CT). The initial weights of the boxes and the weights at the end of the experiment were measured for gravimetric moisture determination. The boxes were incubated on laboratory benches at 24 ± 1 C for the period of the experiment.

Observations. The number of emerged perithecia and pycnidia and the number of sporulated perithecia (that produced ascospores) and sporulated pycnidia (that produced conidia) were counted at 7-day intervals at ×20 in a 1-cm² area on the surface of internodes in contact with the soil. After enumerating the different variables on the internodes, we returned them to the original spatial order in the box. Weekly counts were repeated until the number of perithecia did not increase. Structures with long beaks that protruded from the surface of the internodes were counted as perithecia. Pycnidia appeared as blisters without beaks. Production of ascospores and conidia was marked by the appearance of gelatinous matrices on the perithecia and pycnidia, respectively. To confirm that the ascospores and conidia produced on the internodes were produced by *D. p. caulivora*, the gelatinous matrices from each treatment were streaked on the surface of a selective medium (17) in petri dishes. The dishes were incubated at 24 ± 1 C on laboratory benches, and cultural characteristics were compared with a known culture of *D. p. caulivora*.

Experiment 3. The effects of brief soaking on time and on the number of perithecia and pycnidia that formed and their subsequent sporulation were evaluated in a separate experiment by incubating soaked and unsoaked internodes on soil samples adjusted to the ψ_m treatments described above. Production of perithecia and pycnidia and their respective sporulation were monitored at 7-day intervals.

Experiment 4. The duration of ascospore viability on the surface of perithecia was determined. The pathogen was isolated from the gelatinous matrices at weekly intervals as described above. Isolations were continued as long as *D. p. caulivora* colonies were recovered.

Statistical analyses. Numbers of sporulated perithecia were expressed as percentages of all perithecia present. The number of perithecia formed, the number of sporulated perithecia, and the number of pycnidia formed were transformed as Log_e ($x + 1$) to normalize data before analysis. Repeated measures analysis of variance was used to evaluate the effects of experiment (block), matric potential (treatment), internode (strip plot or repeat measure), day (strip plot or repeat measure), and interactions. The data were analyzed as a strip plot within a balanced split-plot design (16,30), wherein the experiment and ψ_m treatment combinations were the main plot experimental units. The subplot was a strip-plot arrangement of internodes and days. The design treated the internodes and days as equal in the hierarchy, and therefore, either the days can be considered as repeated measures on internodes, or alternatively, the internodes can be considered split within days (15). Huynh-Feldt adjustments were made to all subplot *F* tests to satisfy the assumption that covariance matrices have a type H condition (26).

Linear and nonlinear regression analyses were used to determine the relationship between the formation of perithecia, pycnidia, ascospores, and conidia with soil moisture, and appropriate linear and nonlinear models were fitted. A variety of nonlinear regression models were tested, and we chose the model that best fitted the data. Models were evaluated based on the *F* test, lack-of-fit, and coefficient of determination. All analyses were conducted with SAS General Linear Models procedure (26) and PLOTIT procedure NONL (7).

RESULTS

The relationship between soil moisture and ψ_m in the experiments is shown in Figure 1. Soil moisture varied from 46% at 0.0 MPa to about 10% at -0.32 MPa. Mean water loss during the 42 days of incubation was negligible (0.5% per treatment).

The number of perithecia formed and the number of sporulated perithecia were significantly different in the two experiments ($P \leq 0.05$) (Table 1). However, there was little variation in the percentage of sporulated perithecia between the two experiments. The number of perithecia formed, the number of sporulated perithecia, and the percentage of sporulated perithecia were significantly affected by the moisture treatments, days of incubation, day × treatment interaction, and the relative position of the internodes on the soybean plant (Table 1). The internode × treatment interaction was significant only for the number of sporulated perithecia. The number of sporulated perithecia on the three internodes on different days was significantly different as indicated by the significant day × internode interaction (Table 1). The three-way interaction between day × internode × treatment was significant only for the number of sporulated perithecia (Table 1).

Although the trends in the temporal progress of the number of perithecia formed and the number of sporulated perithecia

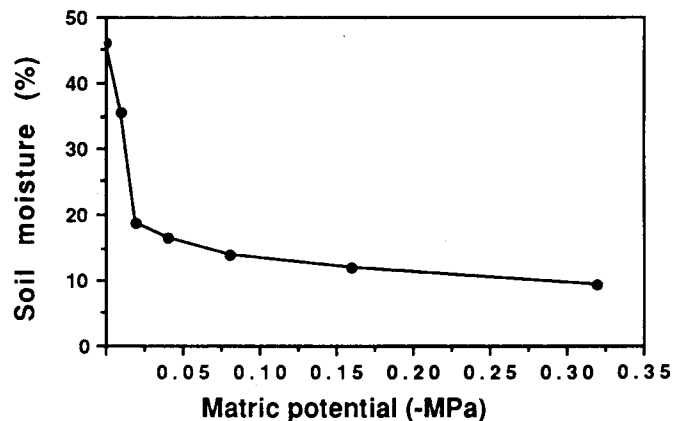


Fig. 1. Relationship between percentage of soil moisture and soil matric potential.

were similar in both experiments, their numbers were significantly different, and therefore, the data are presented for each experiment (Fig. 2).

Formation of perithecia. When the first counts were made, mature perithecia were observed at all matric potential values in both experiments except at -0.32 MPa (Fig. 2A,C). The number of perithecia increased to maximal levels at the second, fourth, and fifth countings in treatments 0.0 to -0.02 , -0.04 , and -0.08 to -0.16 MPa, respectively. At -0.32 MPa, perithecia formed at 7–14 days of incubation, and continued increase in their numbers was observed when counting was discontinued (Fig. 2A,C).

In both experiments, maximum numbers of perithecia formed at a matric potential value of -0.04 MPa, whereas fewer perithecia formed at matric potential values between 0.0 and 0.02 MPa and at -0.08 MPa. At -0.16 MPa, approximately half the number of perithecia that formed at -0.04 MPa developed. Very few perithecia formed at -0.32 MPa (Fig. 2A,C). The number of perithecia at all matric potentials was higher in experiment 1 than in experiment 2.

Sporulation by perithecia. The trends in sporulation by perithecia at different matric potentials were similar in both experiments except at 0.0 and -0.32 MPa. Ascospores were produced by the perithecia in a gelatinous matrix within 7 days at matric potential values from 0.0 to -0.08 MPa (Fig. 2B,D). A linear increase in the number of sporulated perithecia occurred at these matric potential values until 28 days in both experiments, except at 0.0 MPa in experiment 2 (Fig. 2B,D). Further increase in the numbers of sporulated perithecia was observed at -0.08 MPa. At -0.16 MPa, ascospores appeared by 21 days and increased linearly for up to 42 days. At -0.32 MPa, ascospores were seldom produced (Fig. 2B,D) but when produced, were done so after 21 and 28 days in experiments 1 and 2, respectively.

Maximum numbers of sporulated perithecia also occurred at -0.04 MPa in both experiments (Fig. 2B,D). Lower numbers of sporulated perithecia were observed at all other matric potentials. Fewer than 3% of the perithecia that formed at -0.32 MPa produced ascospores (Fig. 2B,D).

Experiment 3. No significant differences occurred in the number of perithecia and pycnidia formed and on the respective spore release of soaked and unsoaked internodes in different treatments. However, soaking reduced the response time of internodes in forming perithecia and pycnidia at different matric potentials. Soaked or unsoaked internodes produced perithecia by 7 or 9 days, respectively.

Experiment 4. The gelatinous matrix had dried at the tip of the perithecia by 14 days at -0.01 to -0.16 MPa. At 0.0 MPa, drying of the gelatinous matrix occurred after 14 days. *D. p. caulivora* was recovered from the dried matrix in isolations made at weekly intervals on a selective medium from all treatments. The pathogen was recovered up to 5 wk after the drying had occurred. Subsequent *D. p. caulivora* isolations were not successful.

Relative position of soybean internodes and the number and sporulation of perithecia. The relative position of internodes on the soybean plant had a significant effect on the number of perithecia that formed, the number that sporulated, and the percentage of sporulated perithecia. Invariably, higher numbers of perithecia formed on the first internode, and lower numbers formed on the third internode in both experiments at all matric potentials (Fig. 3A,C). The differences were most pronounced at -0.04 MPa. Similar differences were also recorded with the number of sporulated perithecia (Fig. 3B,D).

Formation of pycnidia and conidia. Soil moisture did not have a significant effect on the formation of pycnidia (Table 1). Pycnidia also formed by 7 days at matric potential values of 0.0 to -0.08 MPa. However, appearance of pycnidia at -0.16 and -0.32 MPa was delayed by up to 14 days. Formation of pycnidia was erratic and showed no association with the matric potential treatments (data not shown). Compared with perithecia (Fig. 4A), very few pycnidia (Fig. 4C) formed in all of the matric potential treatments. Although variance analysis indicated a significant effect of matric potential treatments on sporulated pycnidia, their numbers were too low for definitive conclusions (Table 1; Fig. 4D).

Relationships among soil moisture and the dependent variables. The relationships among soil moisture, the log-transformed num-

TABLE 1. Analysis of variance for the number of perithecia formed, number of sporulated perithecia, percentage of sporulated perithecia, number of pycnidia formed, and number of sporulated pycnidia in different matric potential treatments in two experiments

Source of variation	df	Mean squares ^x				
		Perithecia formed ^y	Sporulated perithecia ^y	Sporulated perithecia (%)	Pycnidia formed ^y	Sporulated pycnidia
Model	531	1.81**	3.48**	1,689.94**	0.81**	2.17**
Experiment (Exp)	1	62.88**	32.79**	31.76 ^{ns}	11.14*	11.15 ^{ns}
Treatment (Trt)	6	88.96**	223.90**	94,848.74**	3.75 ^{ns}	31.98*
Exp × Trt	6	1.68 ^{ns}	1.13 ^{ns}	22.21 ^{ns}	0.64**	6.24*
Error _a	35	1.28	1.02	107.10	12.81	2.22
Day ^z	5	24.64**	104.22**	146,907.84**	9.59*	5.85**
Day × Exp	5	1.03 ^{ns}	0.26 ^{ns}	61.07 ^{ns}	9.26 ^{ns}	0.96 ^{ns}
Day × Trt	30	9.76**	9.99**	5,217.04**	4.11*	25.36**
Day × Exp × Trt	30	1.17**	0.27 ^{ns}	53.83 ^{ns}	0.61**	1.13 ^{ns}
Error _b (Day)	175	0.64	0.41	181.02	4.24	2.38
Internode ^z (Int)	2	236.02**	137.98**	102.08*	5.39 ^{ns}	20.56 ^{ns}
Int × Exp	2	10.18*	1.41 ^{ns}	13.75 ^{ns}	2.61 ^{ns}	0.39 ^{ns}
Int × Trt	12	3.25 ^{ns}	3.68**	32.79 ^{ns}	2.04 ^{ns}	18.03*
Int × Exp × Trt	12	2.55*	0.75 ^{ns}	16.44 ^{ns}	2.34 ^{ns}	6.17 ^{ns}
Error _c (Int)	70	1.24	0.97	20.82	0.14	18.34
Day × Int	10	19.28 ^{ns}	0.02*	5.02 ^{ns}	0.05 ^{ns}	1.58**
Day × Int × Exp	10	0.22 ^{ns}	0.02*	2.59 ^{ns}	0.10 ^{ns}	0.33*
Day × Int × Trt	60	0.04 ^{ns}	0.03**	3.76 ^{ns}	0.09 ^{ns}	0.82**
Day × Int × Trt × Exp	60	0.07**	0.008 ^{ns}	5.07*	0.18 ^{ns}	0.14 ^{ns}
Error _d (Day × Int)	350	0.02	0.01	3.70	0.00	1.16
Total	881					
C.V. (%)		3.80	3.90	3.67	30.54	220.96

^x ns = *F* tests not significant at $P = 0.05$; * and ** = *F* tests significant at $P = 0.05$ and 0.01, respectively. All tests involving Day and Internode are Huynh-Feldt adjusted *F* tests (26).

^y Variance analysis conducted on $\text{Log}_e(x + 1)$ transformed variables.

^z Repeat measures or strip plots.

number of perithecia formed (Fig. 4A), and the number of sporulated perithecia (Fig. 4B) were nonlinear in both experiments. The second order logistic model significantly ($P = 0.0001$) fit the data from both experiments. The lack-of-fit was not significant ($P = 0.07-0.67$). The models explained 91-96% of the variance in the above variables. The relationship between soil moisture and the percentage of sporulated perithecia in both experiments was linear (Fig. 4E), and the model explained 95% of the variance in that variable. The relationship between soil moisture and the number of pycnidia formed was not consistent in the two experiments (Fig. 4C). Formation of pycnidia and production of conidia were unrelated to the matric potential treatments (Fig. 4C,D).

DISCUSSION

This study demonstrates the influence of soil matric potentials between 0.0 and -0.32 MPa on the formation of perithecia and ascospore production by *D. p. caulivora*. Soil matric potential did not have a pronounced effect on the formation of pycnidia and production of conidia; very low numbers were formed, suggesting that factors other than soil moisture influence the formation of pycnidia and production of conidia.

Maximal numbers of perithecia that formed and the number that sporulated occurred at a matric potential of -0.04 MPa (16% soil moisture) of the matric potentials evaluated. Considerably fewer perithecia formed and produced ascospores at matric potentials of <-0.04 MPa or >-0.04 MPa, indicating that neither low nor excessive soil moisture are conducive for sporulation. The response of *D. p. caulivora* to ψ_m treatments in the range of 0.0 to -0.32 MPa was similar to the effects on many soil fungi (4). Sporulation by perithecia was maximum after

21 days of incubation at soil matric potential treatments of 0.0 to -0.04 MPa, suggesting the greatest availability of primary inoculum occurs after that length of time. In our experiments, at most soil matric potential treatments, formation of perithecia and production of ascospores declined after 28 days of incubation. This decline can be attributed to the exhaustion of perithecia in the infected internodes. In contrast, the formation of perithecia and sporulation in drier treatments (-0.08 to -0.32 MPa) continued to increase, albeit in low numbers. The inoculum produced in such dry conditions may not initiate canker infections, because free moisture is required for spore dispersal and infection (2,5,18). Seasonal variation in stem canker outbreaks has been reported and is due in part to yearly differences in rainfall (2,20,23). Ascospore release generally occurs during April-June and is responsible for primary infections (2). Rainfall during this period initiates sporulation and spore dispersal and provides moisture necessary for infection (2). Thus, delayed planting has been suggested as one of the methods to reduce stem canker infections (2). Results from our experiments also support this view. However, reduced rainfall during April-June and alternate wetting and drying of soil surface in the field may alter the spore release schedules, and delayed planting in those circumstances may not be an effective means of reducing stem canker infections.

Plants used in the study were collected from the field at the end of the soybean-growing season and were stored in the greenhouse (28 C) for 2 wk. Perithecia readily formed on the internodes of these plants, indicating that overwintering is not required for *D. p. caulivora* sporulation. Although experiments were conducted with internodes from soybean plants with seemingly uniform infections, the number of perithecia formed and the number that sporulated were different between experiments. Stem canker infections remain latent for prolonged periods of time, which may result in symptomless plants and symptomless

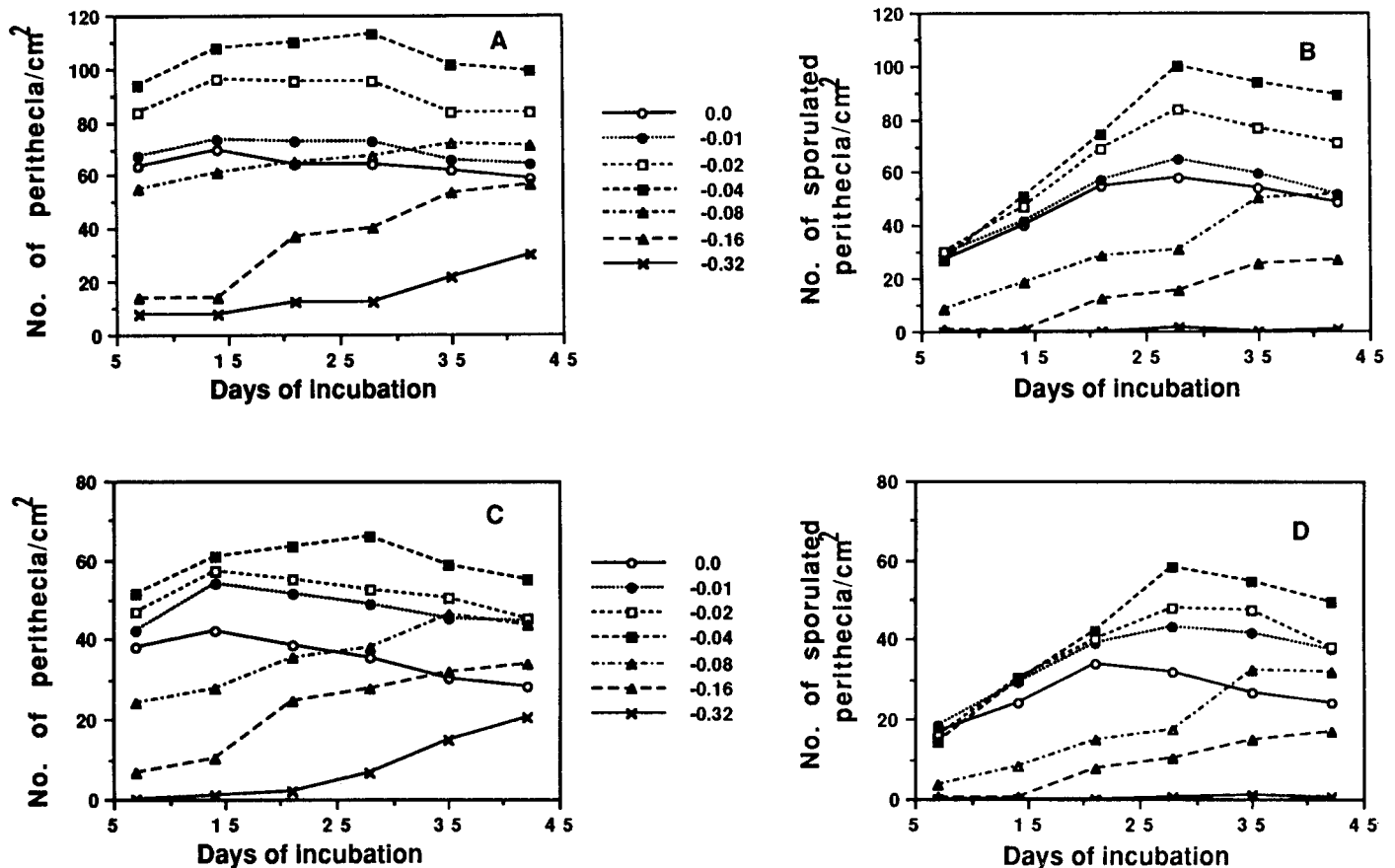


Fig. 2. Effects of various soil matric potentials on A and C, formation of perithecia and B and D, sporulation by perithecia after 0-42 days at 24 ± 1 C. A and B are data from experiment 1; C and D are data from experiment 2.

areas within an infected plant (27). The higher number of perithecia in experiment 1 may have originated from these apparently healthy areas of the internodes.

In the field, perithecia produced on overwintered soybean stem debris provide an important source of primary inoculum (2). The pathogen does not sporulate beneath the soil surface (J. P. Snow, unpublished). Burying the debris by deep ploughing is, therefore, one of the cultural practices followed to prevent infections; only the inoculum produced on the surface of soil initiates stem canker epidemics. Unlike many other soilborne pathogens (4), *D. p. caulivora* always survives on the host debris in the soil. When rainfall occurs, both the soil and the soybean stem debris are soaked simultaneously in the field. In our experiments, these conditions were simulated, and the results were obtained with soybean internodes placed on the soil surface. We also adjusted for the lack of equilibration between the matric potential of the bulk soil and the water status of the soybean internodes by soaking the internodes uniformly before placing them on the soil surface and then counting the number of perithecia on the surface of internodes in contact with the soil surface. Soaking affected neither the number of perithecia and pycnidia formed nor sporulation, but it reduced the time required for equilibration of internode moisture status with the corresponding soil moisture status and thus reflected the true response time of internodes to different soil moisture treatments. However, wetting the internodes may have caused overestimation of the ability of *D. p. caulivora* to

form perithecia at low matric potential values.

The dried gelatinous matrix on the surface of perithecia retained viable ascospores for up to 35 days. The dried gelatinous matrix provides a continuously available source of inoculum for splash dispersal and extends the period of inoculum availability in the field.

The stem and petiole bases are primary courts of *D. p. caulivora* infection (19), and infections occur during vegetative growth stages (5,20). Subsequent progress of the disease occurs by elongation of cankers and girdling of the plants (5,21). In our experiments, more total perithecia and ascospores were consistently formed on the first internode than on all other internodes. The internode that is closest to the soil surface in the field is likely to be infected more frequently, resulting in higher infections; this may explain the increased formation of perithecia on the first internode.

In summary, either low or excessive soil moisture adversely affects the formation of perithecia and the production of ascospores by *D. p. caulivora*. Low moisture conditions after the production of ascospores, however, may prolong the availability of the primary inoculum. In addition to the soil moisture, other factors such as temperature, cultivar, organic matter, pH, and soil potassium content may also account for the variation (22) in the numbers of perithecia formed and in the production of ascospores. Production and sporulation of pycnidia are affected by factors other than soil moisture, and further research is needed to determine the importance of those factors.

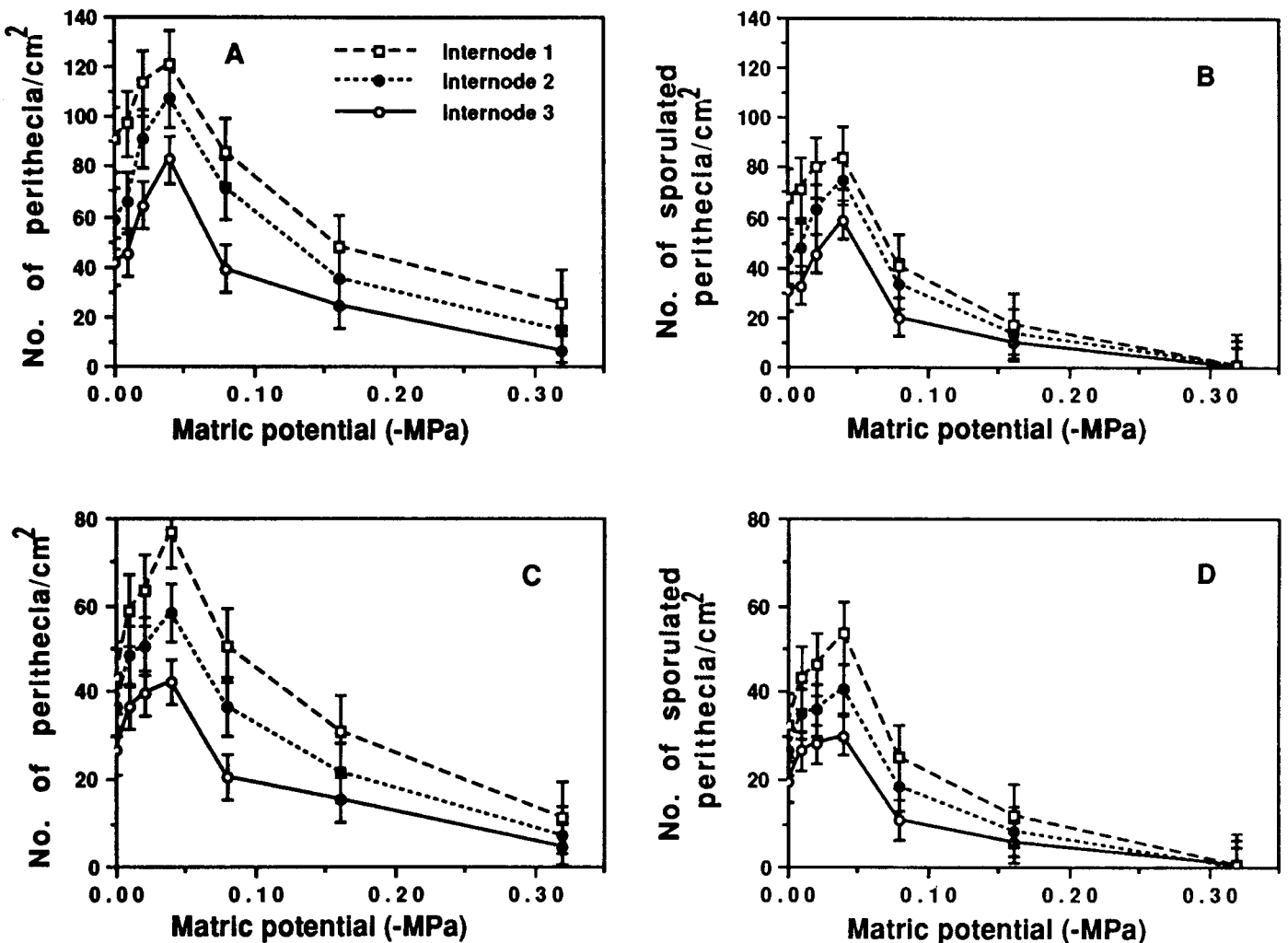


Fig. 3. A and C, formation of perithecia and B and D, sporulation by perithecia on soybean internodes at different soil matric potentials. A and B are data from experiment 1; C and D are data from experiment 2. Numbering of the internodes on the soybean plant was from bottom to top. The first internode refers to the internode on the soybean plant that was closest to the soil surface.

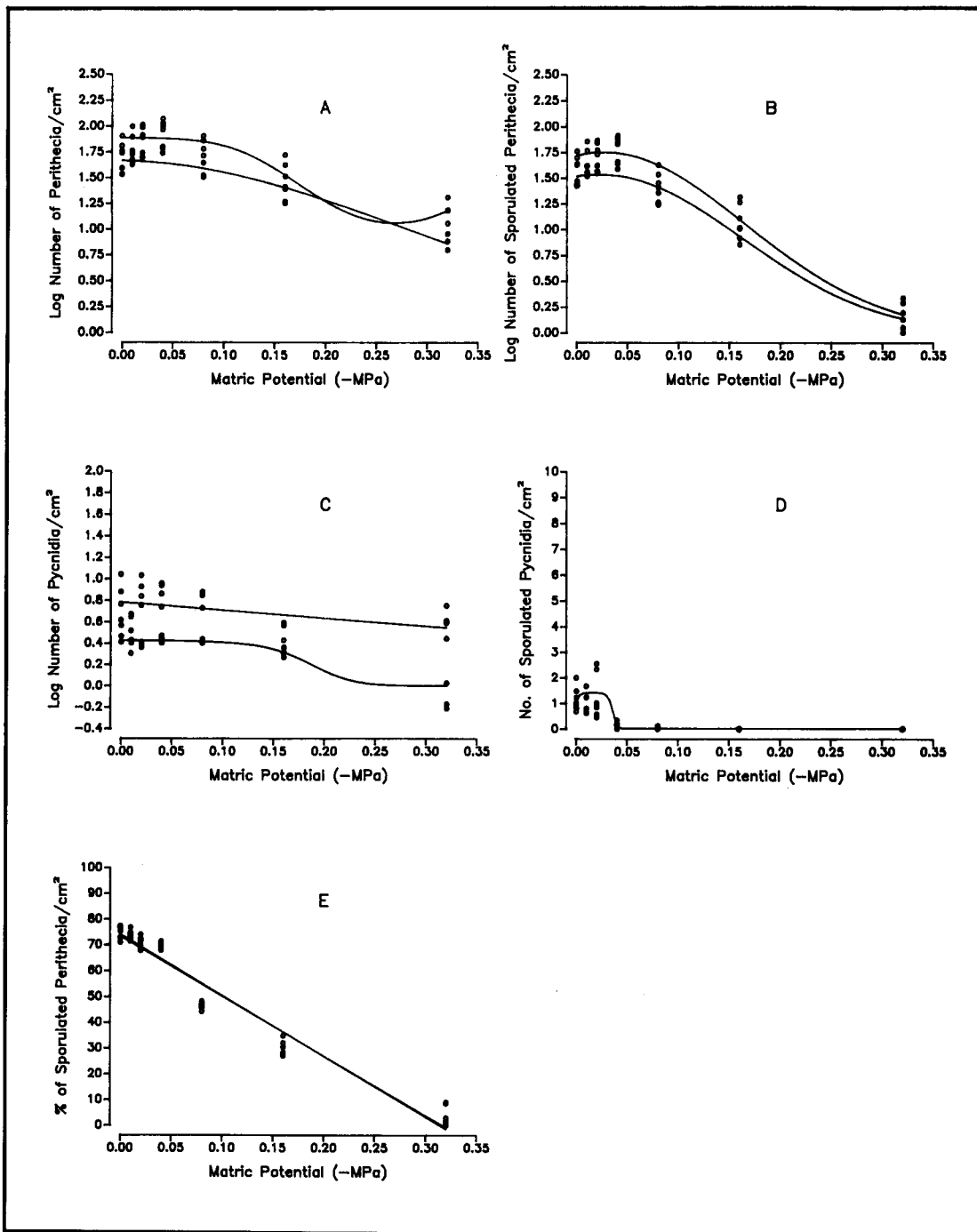


Fig. 4. Relationships between soil matric potentials and A, the number of log-transformed perithecia formed; B, the number of log-transformed sporulated perithecia; C, the number of log-transformed pycnidia formed; D, the number of sporulated pycnidia; and E, the percentage of perithecia producing ascospores. Open and closed circles are the data from experiments 1 and 2, respectively.

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