Effect of Simulated Dew and Postinoculation Moist Periods on Infection of Soybean by *Septoria glycines*

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ABSTRACT

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Experiments were conducted to determine if misting soybean foliage at night to simulate dew formation would affect the severity of brown spot disease. Soybean plants (cultivar Essex) were grown outside in 20-cm-diameter pots and inoculated at flowering with pycnidiospores of *Septoria glycines*. In 1979, lesions induced by *S. glycines* on plants exposed to a 44-hr postinoculation moist period (PMP) were compared to those on plants not exposed to the moist period. Daily simulated dews during the latent infection period caused increased infections on plants not exposed to the PMP. Dews every third or fourth day resulted in increased numbers of lesions on plants exposed to PMP. In 1980, treatments included PMP of various durations, two inoculum levels, and dews daily and every second or third day. Dew exposures again resulted in increased numbers of lesions; increasing the duration of PMP to more than 6 hr occasionally resulted in decreased numbers of lesions.

Additional key words: *Glycine max*.

Recent investigations have demonstrated that brown spot of soybean (*Glycine max* L. Merr.), caused by *Septoria glycines* Hemmi, can cause significant yield losses in certain seasons (1,4–6,13). None of these disease considered the effect of dew on disease development. Previous studies of diseases other than those caused by *Septoria* spp., however, indicate that dew may drastically increase disease severity on aboveground plant parts (12), mainly due to enhancement of spore germination and infection.

The influence of dew on foliar diseases caused by airborne fungal pathogens is well documented (7,8,12). However, little information is available on the effect of dew on diseases caused by pathogens such as *Septoria* spp. whose conidia are disseminated by splashing rain. Because free water is always present during natural dissemination and inoculations with *S. glycines*, it is reasonable to assume that moisture in sufficient for spore germination and penetration. Pycnidiospores of *S. apiicola* Speg. germinate best in free water, but some spores germinate at relative humidities as low as 94.5% when free water is absent (11). Data are not available on the effect of leaf moisture on the development of infection by *S. glycines*. Previous results have shown that the latent infection period may extend for 3–4 wk in plants of soybean cultivar Essex inoculated during flowering (13). This latent period was chosen as the period to expose the inoculated plants to various dew periodicities because latent periods of *S. nodorum* on wheat were found to decrease with increased durations of leaf wetness (10).

Because brown spot of soybean only sporadically causes measurable yield losses in North Carolina (6,13), this study was initiated to determine the effects of artifically induced dews (leaf wetness periods simulated by exposing foliage to intermittent mist generated at night) on the development of this disease. The objective of this research was to determine the effect of various postinoculation leaf wetness periods on the number of brown spot lesions that subsequently develop on inoculated soybean leaves.

MATERIALS AND METHODS

The isolates of *Septoria glycines* used in these experiments were collected in North Carolina in 1975. Pycnidiospores were produced on PDA, harvested, and prepared for inoculum as previously stated (13).

Seeds of cultivar Essex were planted in a sandy loam in 20-cm-diameter pots on 21 May 1979 and 26 May 1980. After emergence, the plants were thinned to three per pot. Pots were located outdoors

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throughout the experiment (except during incubation in a mist chamber immediately following inoculation) on a raised sand bed (1.2 × 51 m) and were fertilized periodically with a complete fertilizer (VHPF, Miller Chemical and Fertilizer Corp., Hanover, PA).

Abaxial leaf surfaces were sprayed until runoff on 7 August 1979 and 24 July 1980 with inoculum delivered from a CO_2-powered sprayer at 2.1 kg/cm^2. At these dates, flowering had almost ceased in 1979 and was midway in 1980. In 1979, all expanded leaves were inoculated, and in 1980 only the fully expanded leaves on the main axis were inoculated.

In 1979, all plants were inoculated with 1×10^5 spores per milliliter and the experimental design was a 2 × 5 factorial. Inoculated plants either were not incubated or were incubated in a mist chamber for 44 hr at 24–29 C, and there were five different dew frequencies. Each dew treatment with incubated plants had three replications (pots) and the nonincubated plants had two replications per dew treatment. After incubation, the pots were plunged into the sand bed in circles of five pots each ~90 cm in diameter (outside); each group was centered at 180-cm intervals and centered under a fog-mist nozzle. All pots in the same dew treatment were located under the same fog-mist nozzle.

The experimental design in 1980 was a $2 \times 4 \times 4$ factorial: two inoculum concentrations (1×10^5 and 3×10^4 spores per milliliter); four different moist periods (6, 12, 24, and 36 hr) following inoculation; and four different periods of dew formation. Each treatment at the lower inoculum level had three replicate pots, and treatments at the higher level had one pot. After each moist incubation period (23–27 C), the foliage was allowed to dry in the greenhouse and pots then were arranged in the sand bed in eight groups of eight pots each under fog-mist nozzles in a fashion similar to that of 1979. The four pots in each dew exposure schedule were divided equally between two separate fog-mist nozzles.

In 1979, dews were initiated 9 August and continued for 24 days with the following treatments: nightly (25 times); every other night (12 times); every third night (eight times); every fourth night (six times); and no dew. In 1980, dews were initiated on 25 July and continued for 24 days as follows: nightly (25 times); every other night (13 times); every third night (nine times); and no dew. Average daily maximum and minimum temperatures during dew exposure periods were 31.6 and 18.8 C in 1979 and 33.8 and 22.3 C in 1980.

Data on the average number of lesions per leaflet of each trifoliolate leaf on the main stem were recorded between 8 and 11 September of both years. Disease indices, based on an estimation of the number of lesions per leaflet at nodes seven to 11 in 1979 and seven to 12 in 1980, were: 1, no lesions; 2, 10 lesions; 3, 20 lesions; 4, 30 lesions; and 5, ≥50 lesions. Decimals were used for intermediate values. The data were subjected to analysis of variance. The 1980 data from plants inoculated with higher and lower inoculum densities were analyzed separately.

Mist system. A polyvinyl chloride pipe (1.25-cm diameter) with a fog-mist nozzle teed off the main line via a gate valve above each group of plants was supported down the center of the sand bed 15–20 cm above the uppermost leaves. The main pipe was plugged at one end, and the other end was fitted with a solenoid valve that was connected to a source of deionized water pressurized at 3.51 kg/cm^2 by a CO_2 cylinder. The solenoid valve was controlled by a mercury switch fitted with an adjustable, balanced, horizontal wire-mesh screen located just above the leaves in one group of plants that received dew daily. The mercury switch was adjusted so that when the screen was dry, the solenoid valve opened and a fine mist was emitted from each nozzle that was turned on at that time. The weight of the water caused the screen to activate the mercury switch, which energized the solenoid and shut off the water. As the wire mesh dried off, the system again rewet the foliage. The drying of the wire screen approximated that of the soybean foliage. The electrical power for the system was wired to a time clock that turned on the current at night between 2000 and 0800 hours.

During the period of dew exposures, the entire bed was surrounded by a wire-mesh screen 120 cm high to minimize wind effects on the mist applications. Each night a double thickness of cheesecloth was placed over the groups not receiving moisture to prevent the formation of natural dew (7). No effort was made to shield any of the plants from rain.

RESULTS

Small lesions had appeared 17–20 days after inoculation (30 August in 1979 and 14 August in 1980) and by 9–11 September some lesions had reached maximum size and a few leaves were chlorotic and abscised.

1979. Although average disease indices indicated that exposure to certain simulated dew periodicities caused an increase in lesions, the large coefficient of variation (105%) prevented the differences among the data from dew treatments within either postinoculation moist period from being statistically significant (Table 1). However, among the plants given postinoculation moisture, all dew exposures caused higher disease indices than those observed on plants not exposed to dew. There was a significant difference ($P = 0.01$) in disease severity between treatments exposed to postinoculation moisture (average disease index = 3.0) and those not exposed to this moist period (1.9). Of the plants not given a postinoculation moist period, those exposed to dew every night had the highest disease index. Average disease indices were higher for lower nodes than the upper nodes; ratings from lower to upper nodes were 2.7, 2.7, 2.4, 2.4, and 2.2.

1980. Average disease indices were greater for plants inoculated with the higher inoculum level than for plants in the same treatment inoculated with the lower inoculum level (Table 1). Plants exposed to daily dews had more disease than plants not exposed to dew at either inoculum level. This was most noticeable on plants inoculated with the lower inoculum level and incubated for 6 hr. Decreasing the dew frequencies or increasing the length of postinoculation moist periods of plants inoculated with the lower inoculum level resulted in decreased disease in some treatments. These trends were not as evident on plants inoculated with the higher inoculum level. As in 1979, overall disease ratings for the lower nodes were greater than those for the upper nodes. Average ratings for the lower inoculum level treatment from lower to upper nodes were 2.1, 2.2, 2.0, 1.9, 1.4, and 1.1, and for the higher inoculum group were 3.3, 3.4, 3.4, 3.2, 2.7, and 2.2.

DISCUSSION

The marked increase in numbers of brown spot lesions in response to periods of simulated dew indicates that disease severity could be increased by natural dews during postflowering stages under field conditions. Since all plants were grown outside during the "normal" season and developed as field-grown plants, the results are more nearly comparable to those that would be obtained under natural conditions than those that have been obtained with plants grown in the greenhouse or growth chamber (2,9,10).

The 1979 data demonstrated that both a postinoculation wet period and (probably) dew exposures promote brown spot development. However, with only one group (location) used for each dew exposure, the effect of location on disease development could not be separated from that of dew exposures. The use of two separate groups in 1980 for each dew frequency permitted evaluation of this possible effect.

The trend in 1980 for longer postinoculation wet periods to either not affect, or (at times) decrease, lesion numbers is opposite to the trend reported with *S. nodorum* on wheat (2,9,10). Extended postinoculation moist periods may have washed some spores off the foliage and removed a greater proportion of inoculum from leaves inoculated with the lower level than with the higher level. This could account for the greater decrease in disease with increasing postinoculation wet periods obtained with the lower inoculum compared to the higher inoculum. This effect could also reflect inoculum mortality or inhibition caused by microbial antagonists present on outdoor-grown soybean foliage exposed to prolonged moist periods (3). Plants grown inside growth chambers (2) probably would not have epiphytic microbial populations similar to those on plants grown outside (3).

TABLE 1. Average brown spot disease indices of soybean cultivar Essex leaves at nodes seven through 12 exposed to various dew frequencies and postinoculation moist periods immediately following inoculation with *Septoria glycines*[w]

Postinoculation moist period (hr)	Dew frequency[x]				
	0	Daily	Every second day	Every third day	Every fourth day
1979					
Higher inoculum[y]					
0	1.8	2.7	1.6	1.7	1.8
44	2.4	3.0	2.7	3.6	3.5
1980					
Lower inoculum[yz]					
6	1.52 bc	3.08 g	1.92 cd	2.03 ef	···
12	1.20 a	2.12 ef	1.37 ab	1.53 bc	···
24	1.63 bc	1.88 de	1.73 cd	1.56 bc	···
36	1.45 ab	1.77 cd	1.75 cd	1.23 a	···
Higher inoculum[yz]					
6	2.52 b	3.92 ef	3.72 def	3.41 def	···
12	2.65 b	3.61 def	2.73 bc	3.22 d	···
24	3.35 de	3.95 f	3.27 cd	···	···
36	1.66 a	3.92 ef	2.12 ab	3.54 def	···

[w] Average disease severity indices were based on numbers of lesions per leaflet: 1 (none); 2 (10); 3 (20); 4 (30); 5 (50 or more lesions).
[x] Dew was applied for 25 days between 2000 and 0800 hours after an initial postinoculation moist period.
[y] Lower inoculum = 3×10^4 spores per milliliter; higher inoculum = 1×10^5 spores per milliliter.
[z] Means not followed by the same letter within each row are significantly different ($P = 0.05$) according to Student's t-test of adjusted means. Pots within moist period × dew frequency were used as the error for the low inoculum, and plant-to-plant variation within a treatment combination was used as error for the high inoculum.

The increased infections that occurred in 1980 with increasing dew frequencies even after prolonged postinoculation moist periods could be a response to delayed or erratic germination of the pycnidiospores. Sheridan (10) obtained erratic germination with inoculum of *S. apiicola* produced on PDA. If, under natural conditions, all spores dispersed by splashing rain are not at a germinable stage and germinate only after a period of time on the leaf surface, then subsequent dews would enable these spores to germinate and increase the number of infections. Other possible explanations for the increased lesion numbers associated with leaf wetness may reside in changes of internal leaf environment or host physiology favorable for disease development after or during stomatal penetration. Histological or physiological studies are required to explore these possibilities.

The greater number of lesions observed on leaves at lower nodes (older leaves) verifies field observations that diseased plants defoliate acropetally. Because dews wet leaves in the uppermost canopy more than the sheltered leaves below, this moisture and any subsequent increase in brown spot on the more photosynthetically active upper canopy could make the frequency of dew a very important factor in determining whether the disease causes a yield loss. Further field studies of the brown spot disease are needed to elucidate the enhancing effect of leaf moisture. Utilization of dew frequency data along with those for cultural and other environmental factors may enable forecasting of highly probable times for brown spot to cause yield losses and perhaps the location of geographic areas with high yield loss potential.

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