Effects of Temperature and Leaf Wetness Period on Brown Spot Disease of Soybeans

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ABSTRACT

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The effect of temperature and leaf wetness period on the development of *Septoria glycines* infection of soybean (*Glycine max*) leaves in growth chambers and in vitro was studied under controlled environmental conditions. A minimum relative humidity of 95.6% was necessary for conidial germination and vegetative growth on potato-dextrose agar. On water agar (1.5%), more than 50% germination occurred at 24–32 C after 24 hr of incubation and more than 80% germination at 16–32 C after 36 hr. Similar germination percentages at 24 and 36 hr of incubation occurred on soybean leaf disks at 24–28 C and 20–28 C, respectively. No germination occurred at 36 C on either water agar or soybean leaf disks. Maximum germ tube growth of 667 μm occurred on water agar at 28 C after 36 hr. Germ tubes from 145 to 180 μm long developed on leaf disks after 36 hr between 24 and 32 C. A minimum leaf wetness period of 72 hr was required for leaf infection, but a longer period accelerated fungus development. Leaf infections occurred between 16 and 32 C, with optimum disease development at 28 C.

Brown spot disease of soybeans (*Glycine max* (L.) Merr.) is a major foliar disease of soybean. *Septoria glycines* Hemmi, the causal organism that was first described in 1914 (4), causes reduction of the photosynthetic area through destruction of leaf tissue and induction of premature senescence of severely infected leaves. Brown spot has been reported in almost all countries where soybeans are grown (10). Reductions in yield have been reported in field inoculation studies involving brown spot (6,14).

Development of brown spot is most severe in damp, warm weather and has been reported as epiphytotic following protracted rainy periods (12). The incidence of brown spot decreases, and the disease is usually absent on the upper leaves in midsummer (9,13). The effect of environmental factors on the development of plant diseases is important in the evaluation of cultivar resistance to pathogens and the development of other control methods. The objectives of this research were to determine the minimum relative humidity (RH) needed for conidial germination and vegetative growth, the effect of temperature on the percentage of germination and germ tube length, and the interaction of leaf wetness period (LWP) and temperature on disease development.

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MATERIALS AND METHODS

Culture isolation and inoculum preparation. *S. glycines* was isolated from naturally infected soybean leaves. Eight to 10 days before inoculum was required, conidia from a stock culture were streaked on potato-dextrose agar (PDA) and incubated at 25 C under 15 hr of light in a growth chamber (110 μE/m^2 per second). Conidia were collected by flooding the slants with sterile, deionized water. The surface of the culture was rubbed lightly with a sterile transfer loop to loosen the conidia. The conidial suspension from two or three slants was strained through four layers of cheesecloth and collected under suction filtration on the surface of a Nalgene (Millipore Filter Corp.) 20-μm membrane filter unit. Sterile, deionized water was added twice and filtered through to wash the conidia. The conidia were resuspended in a sterile 0.02% water solution of Tween 80 (polyoxyethylene sorbitan mono-oleate, U.S. Biochemical Corp.). The concentration of the conidia was determined with a hemacytometer and adjusted to that needed for the specific experiment by the addition of sterile 0.02% Tween 80.

Plant materials and growth conditions. The soybeans (cv. Woodworth) were

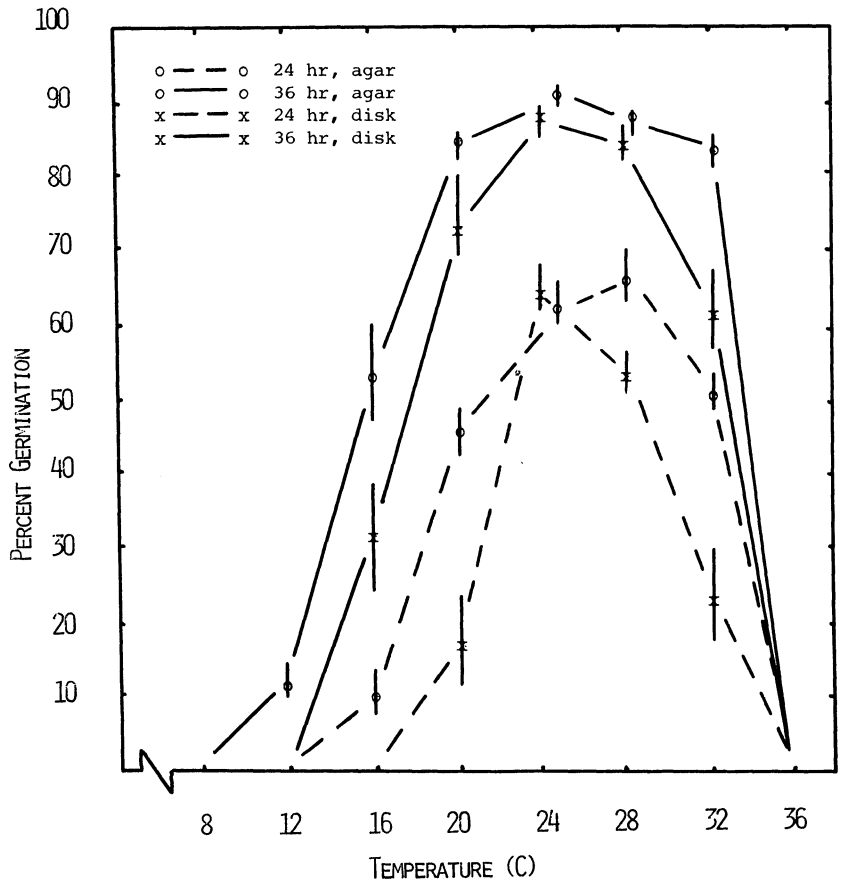

Fig. 1. Percentage of germinated conidia of *Septoria glycines* after 24 and 36 hr on 1.5% water agar or soybean leaf disks at temperatures from 8 to 36 C.

grown in 6.5-cm-diameter plastic pots in a mixture of perlite and vermiculite (1:1) in growth chambers with 15 hr of light (110 $\mu E/m^2$ per second). Plants were inoculated or leaf disks taken when the first trifoliate leaf was fully expanded, approximately 16 days after sowing.

Conidial germination. A modified technique of MacNeill (7) was used to study the effect of relative humidity on germination of conidia. The open-faced bottoms of two 9-cm petri dishes were placed together and the edges sealed with a wide rubber band. The lower plate contained a sulfuric acid solution of known concentration (11), and the top plate contained a thin layer of solidified PDA. A series of solutions was prepared to give a range of relative humidities from 89.9 to 100%. The sealed plates were kept at 25 C for 12 days to allow for equilibration of the atmosphere before inoculation of the upper plate with 0.5 ml of conidial suspension containing 10–100 conidia and incubation in darkness at 25 C for 5 days. The experiment was repeated four times.

The effect of temperature on conidial germination and germ tube growth from 8 to 32 C in 4-C intervals was examined on 1.5% water agar (WA) and on 11-mm-diameter soybean leaf disks. A thin layer of WA was placed in 5-cm petri dish halves that were then enclosed in 9-cm petri dishes. The larger petri dishes contained a small amount of water to maintain a high degree of relative humidity within the containers. Using a microliter syringe, 100–150 conidia suspended in 5-μl droplets of 0.02% Tween 80 water solution were placed in the center of the 5-cm dishes. The percentage of germination and germ tube length was determined after 24 and 36 hr of incubation for all temperatures and also after 48 hr of incubation for the trials at 12 and 16 C. The conidia were stained with aniline blue in lactophenol, covered with a coverslip, and observed directly under the light microscope.

The percentage of germination was estimated by observing 100 conidia in each petri dish. Germ tube length of 10 conidia on each petri dish was determined using a calibrated ocular micrometer. The length of the conidium was included in the measurement. Three dishes were observed at each temperature and incubation period tested. The experiment was repeated four times.

Leaf disks were floated (lower epidermis up) in sterile, deionized water in 9-cm petri dishes. Germination percentage and germ tube length were determined after 24 and 36 hr. Leaf disks were dried under a heat lamp to remove water from the surface, after which conidia were removed from the leaf surface using a thin-layer Parlodion technique. A drop of Parlodion (Mallinckrodt Chemical Co.) solution (1 part Parlodion in 20 parts of a 3:1 mixture of ether and ethanol) was spread on the surface of the leaf disk. After the solvent had evaporated, the Parlodion layer was peeled off and placed on a microscope slide. The Parlodion peel was stained with 0.05% trypan blue in lactophenol, covered with a coverslip, and observed under the light microscope. Conidia on three peels were observed at each temperature and incubation combination tested. The experiment was repeated three times.

Infection of soybean plants. The lower surface was sprayed with a conidial suspension (10^5 conidia per milliliter of 0.02% Tween 80) when the first trifoliate leaf was fully expanded. The plants were placed in dew chambers in a growth chamber at least 4 hr prior to inoculation. Temperature within the dew chamber was 2 C higher than the temperature of each growth chamber. After inoculation, the plants were placed back in the dew chamber and incubated at the desired temperature. Four plants were removed from the dew chamber at 24-hr intervals for 5 days and placed in the same growth chamber, which had an RH of 60%. Disease ratings were recorded 8 days after inoculation. The experiment was repeated four times.

The effect of temperature and LWP was determined at 16, 20, 24, 28, and 32 C for LWP of 72 and 96 hr. Immediately following inoculation, 12 plants were incubated in dew chambers at the experimental temperature. Six plants were removed after 72 hr and incubated in another growth chamber at 26 C. The remaining six plants were removed after 96 hr and incubated in the growth chamber with the preceding six plants. The experiment was repeated three times at each temperature. Each time the experiment was run, three or four plants were inoculated and used as checks. The check plants were incubated in a dew chamber for 96 hr at 28 C, then with the experimental plants at 26 C. Plants were rated for disease 8 days after inoculation.

Disease rating. Disease development was rated by superimposing a clear plastic grid over the upper surface of the leaf. The grid had large squares with an area of 1 cm^2 each, which was divided into 25 smaller squares with areas of 4 mm^2. The number of small squares that contained necrotic tissue was recorded. Scores ranged from 0 to 25. Every large square completely overlaying leaf tissue was scored. An average was computed for each leaf and used to compute a mean score for the trial.

The raw data within each trial were converted to a disease index designed after that of Marchetti, Melching, and Bromfield (8). The disease index was computed by dividing the disease rating

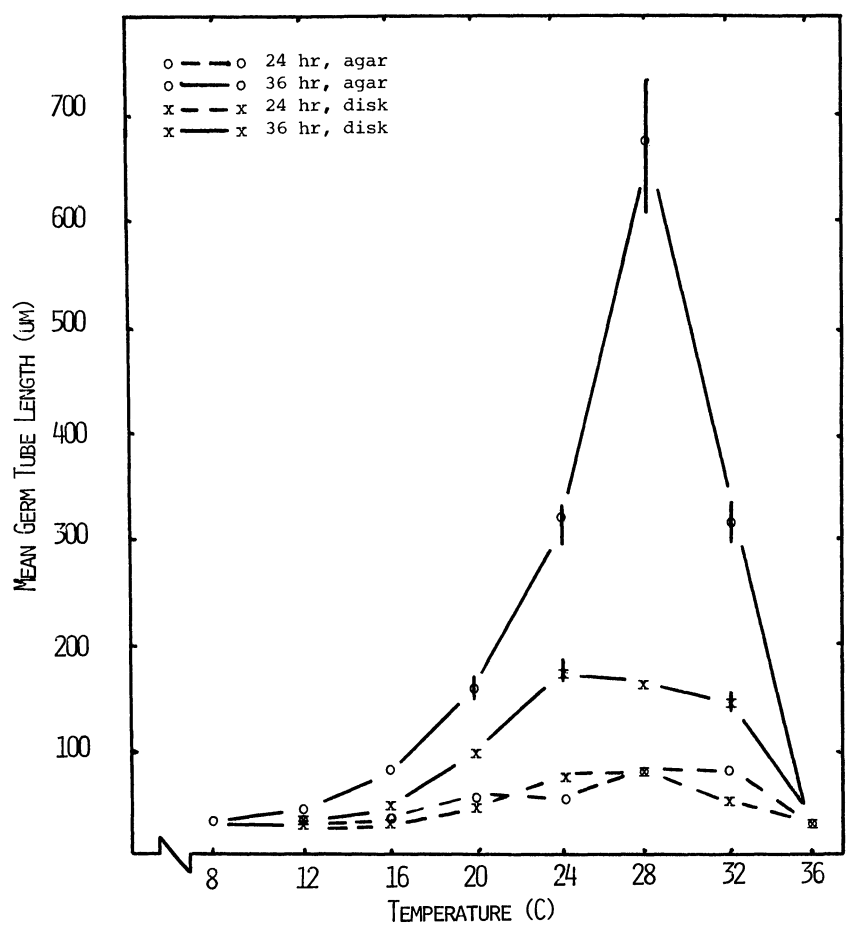

Fig. 2. Length of germ tubes developing from conidia of *Septoria glycines* after 24 and 36 hr on 1.5% water agar or soybean leaf disks at temperatures from 8 to 36 C.

score of the experimental plants in a trial by the disease rating score of the control plants run in the same trial. This allowed for comparison among results obtained in all trials. Comparison of direct scores was not valid because of day-to-day differences in infectivity of inoculum and other uncontrolled factors. The scores of the controls among trials varied from 3.56 to 15.01 small squares (each 4 mm^2) containing diseased tissue per large square area of 1 cm^2. The Kruskal-Wallis test (1) was used to determine whether the disease indices differed significantly. This test was used because the data were not normally distributed.

RESULTS

Effect of humidity on conidial germination and vegetative growth. Conidia germinated and produced colonies within 5 days at all RHs tested above 95.6%, but with inconsistent germination and vegetative growth occurring between 97.5 and 95.6% RH. Germination and vegetative growth did not occur below 95.6% RH.

Effect of temperature on conidial germination and germ tube growth. Conidia germinated in the range of 16 to 32 C on both WA and leaf disks after 24 hr of incubation (Fig. 1). Germination occurred after 36 hr in the range of 12 to 32 C on WA but remained in the 16–32 C range on leaf disks. Increasing the incubation time from 24 to 36 hr increased germination 20–40% on WA and 20–60% on leaf disks. The incubation period increased to 48 hr for the 12 and 16 C treatments on WA, which resulted in a further increase in germination to 38 and 80%, respectively.

Moderate germination (40–70%) occurred in the range of 20 to 32 C after 24 hr on WA. Higher percentages of germination (82–90%) occurred after 36 hr of incubation in the range of 20 to 32 C. At both 24 and 36 hr, germination dropped rapidly outside the range of 20 to 32 C. After 24 hr, moderate germination (50–70%) occurred at 24–28 C but did not drop rapidly until 16 and 32 C. In all cases, germination ceased between 32 and 36 C. Variation in germination between and within replicates was low at favorable temperatures (20–30 C) and higher outside this temperature range.

Little germ tube growth occurred after 24 hr on both WA and leaf disks (Fig. 2). Mean germ tube length did not exceed 90 μm at any temperature. On WA, germ tube growth had a maximum at 28–32 C, whereas on leaf disks the maximum growth was at 24–28 C. After 36 hr on WA, germ tube length increased rapidly from 43 μm at 12 C to a maximum of 667 μm at 28 C. Beyond 28 C, germ tube growth was reduced and ceased between 32 and 36 C. (Because the conidial length was included in the measurement, no growth is represented by 30 μm, the average length of the conidium, in Figure 2.) Germ tube growth was considerably less on leaf disks as compared with WA after 36 hr of incubation. Maximum germ tube growth (140–180 μm) occurred in the range of 24 to 32 C after 36 hr on leaf disks. Variation in germ tube length between and within trials was greatest during this favorable temperature range.

Effect of LWP on infection and disease development. Little disease developed during LWPs of 48 hr or less at 28 C (Fig. 3). The direct scores of the 24-hr trials varied from 0.06 to 0.67 small squares (4 mm^2) containing necrotic tissue per large square area. The disease rating increased after 48 hr of LWP, with direct necrotic area scores varying from 0.94 to 2.80. The period between 48 and 72 hr was critical for development. The disease index increased sharply during this period, and direct necrotic area scores ranged from 4.96 to 9.37. Increasing the length of LWP beyond 72 hr continued to increase the disease rating. After 120 hr of LWP, the direct scores varied from 9.03 to 17.53. LWP beyond 120 hr gave disease development in which the entire leaf curled or senesced before the 8-day postinoculation incubation period ended. Data from this study, analyzed using the Kruskal-Wallis test (1), and infection index between time intervals were found to be significantly different at $P = 0.01$.

Effect of temperature and LWP on disease development. Disease developed at all temperature-LWP combinations tested (Fig. 4). The extent of disease increased steadily from 16 C to an optimum at 28 C. Beyond 28 C, the amount dropped rapidly to almost 0 at 32 C. Extending the LWP from 72 to 96 hr increased the disease rating to about the same extent as did temperature increase except at 32 C. The disease indices at 72 hr LWP were significantly different at $P = 0.05$ and at $P = 0.01$ for a 96-hr LWP.

DISCUSSION

A minimum RH of 95.6% was necessary for conidial germination and growth of *S. glycines* on PDA, but free water was not necessary for germination as it is for many other fungi (2,5). Germination and growth occurred within a temperature range of 12 to 32 C.

Few lesions developed with a LWP of 48 hr or less in inoculated plants in dew chambers. A minimum LWP of 72 hr was necessary for good disease development at 28 C, the optimum temperature for germination and germ tube growth. Disease continued to increase in LWP beyond 72 hr. Under field conditions, the length of periods of favorable RH is a limiting factor in disease development (9). The epiphytotic outbreaks of brown spot following protracted rainy periods reported by Wolf and Lehman (12) can be attributed to the resulting high RH, which allows for conidial germination

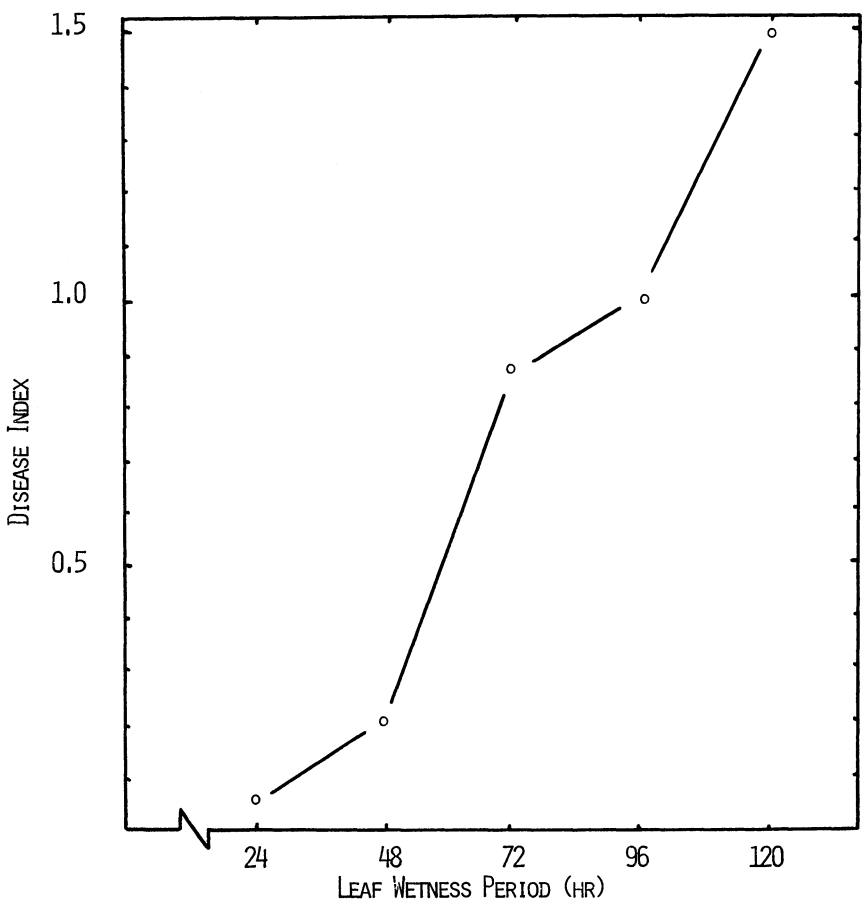

Fig. 3. Relation of disease index recorded for Woodworth soybean leaves infected by *Septoria glycines* at 28 C to leaf wetness period.

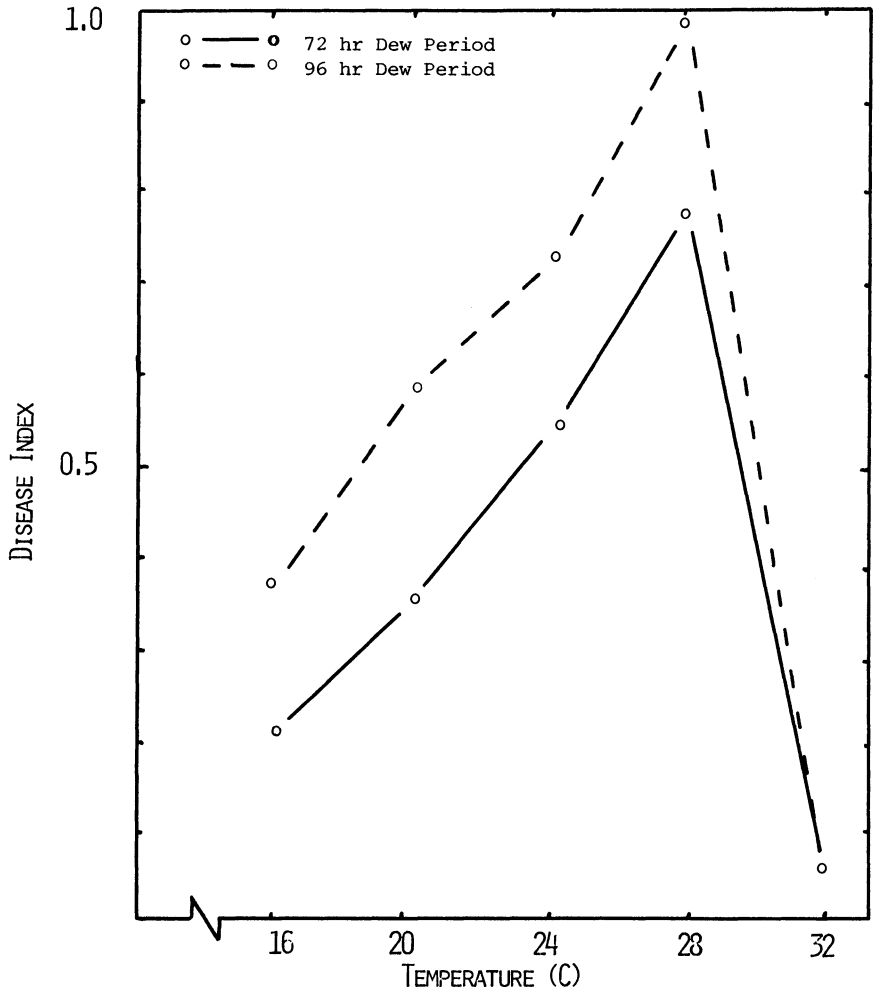

Fig. 4. Relation of disease index recorded for Woodworth soybean leaves infected by *Septoria glycines* at 72- and 96-hr dew periods to temperatures from 16 to 32 C.

and germ tube growth. High RH is required by *Septoria* spp. for extrusion of conidia from pycnidia (3). If this is true for *S. glycines*, high RH would then provide conidia for further spread of brown spot.

Brown spot did not develop at temperatures above 32 C. Such temperatures are common in midsummer in central Illinois. This may explain the observations (9,13) that the disease is absent on upper leaves during midsummer. The additional requirement of 72 hr of RH above 95.6% is rarely met during midsummer in most soybean-growing areas.

ACKNOWLEDGMENT

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