CANADIAN JOURNAL OF PLANT PATHOLOGY 12: 25-30, 1990

Effect of temperature and light on sporangial germination and zoospore infectivity in *Sclerospora graminicola* on pearl millet

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Accepted for publication 1989 12 12

The effects of temperature on germination of *Sclerospora graminicola* sporangia (zoospore release), zoospore viability, and germ tube growth, and of light and temperature on zoospore infectivity and downy mildew development in pearl millet (*Pennisetum glaucum*) were studied. Germination of sporangia was highest at about 30° C and little germination occurred after 2 h 40 min. Germ tubes grew at 15–35° C. Zoospores retained high infectivity (≥ 70% systemic infection) for 4 h at 30° C, and for a longer time at lower temperatures. Zoospore infectivity was not affected by light. The shortest incubation period (3 days) and the highest infectivity (66%) were obtained at 30° C. Although both roots and shoots of young seedlings were susceptible, incidence of downy mildew was highest when both were coinoculated.

Singh, S.D., and **R. Gopinath, 1989.** Effect of temperature and light on germination of sporangia and infectivity of zoospores of *Sclerospora graminicola* on pearl millet. Can. J. Plant Pathol. 12: 25-30.

Nous avons étudié les effets de température sur la germination des sporanges de *Sclerospora graminicola*, la viabilité des zoospores et la formation du tube germinatif. Les effets qu'ont la lumière et la température sur l'infectiosité des zoospores ainsi que le développement du mildiou chez le mil (*Pennisetum glaucum*), ont été également étudiés. La germination a atteint son apogée à 30° C mais a beaucoup diminué après 2 heures 40 min. Les tubes germinatifs ont été formés entre 15 et 35° C. Les zoospores ont présenté une forte infectiosité (≥ 70% d'infection systémique) à 30° C pendant 4 heures. Plus la température était basse, plus la durée de rétention était longue. Cette infectiosité n'était pas affectée par la lumière. Ainsi, nous avons pu obtenir la plus courte période d'incubation et la plus forte infectiosité à 30° C. Malgré le fait que les racines et les pousses des plantules étaient sensibles, la plus forte incidence de mildiou a été enregistrée quand les deux étaient inoculées ensemble.

The role of sporangia in the epidemiology of downy mildew (DM) [*Sclerospora graminicola* (Sacc.) Schroet.] of pearl millet [(*Pennisetum glaucum* (L.) R. Br.] has been controversial for many years (2,4). Before the biology of sporangia was documented, researchers believed that sporangia did not play much of a role in disease epidemiology (2). Recent studies have clearly established the importance of sporangia in the epidemiology of this disease (5,8), and sporangia are now being utilized largely for resistance screening in the greenhouse, laboratory, and the field (6,11).

To further improve screening techniques, and for precise pathological studies, more knowledge of the biology of sporangia is needed. The effects of environmental factors on the production of sporangia have been described recently (7). During the period from production and infection, sporangia undergo several processes that are directly influenced by environment, particularly temperature and relative humidity (RH). The purpose of this study was to investigate the effects of temperature and light on sporangial germination, germ tube growth, and development of systemic symptoms.

Materials and methods

Host cultivar. A highly susceptible pearl millet landrace, 7042 (IP 2696) from Chad, which normally develops > 80% DM incidence under moderate disease pressure, was used throughout this study. For all experiments, seeds (surface-sterilized with 0.1% $HgCl_2$ for 5 min, followed by several rinses in sterile distilled water) were germinated in incubators at 25-30° C in a 12-h-light/12-h-dark cycle.

Preparation of inoculum. Downy mildew inoculum was obtained from systemically infected leaves of plants grown in a greenhouse. These plants were infected by the ICRISAT Center isolate of the pathogen. Leaves were excised, washed with water to remove old spores, and incubated at 20° C in humidity chambers (100% RH). After 6 h incubation, sporangia were collected by washing the leaves with cold (2-4° C) tap water. The sporangial concentration was determined using a hemacytometer; the inoculum was then adjusted to the desired concentration, and used immediately.

Effects of temperature on germination of sporangia and germ tube growth. Samples (5 mL) of a sporangial suspension (1×10^5 sporangial mL^{-1}) were placed in 25-mL beakers and incubated at 10, 15, 20, 25, 30, 35, 40, and 45° C (six beakers per temperature). To determine germination of sporangia, one beaker was removed from each temperature treatment every 30 min and a few drops of lactophenol were immediately added to each sample to stop further germination. Using a

hemacytometer, the number of germinated sporangia (empty sporangial sacs) and total sporangia were determined in 12 microscope fields (125 ×) for each sample, and an average was calculated. The experiment was done twice.

For measuring germ tube growth (length), a dilute suspension of zoospores was placed on hemacytometer slides, and the locations of isolated zoospores were noted. Four slides were incubated at each of five temperatures (15, 20, 25, 30, and 35°C) in petri-plate humidity chambers. Effect of one temperature treatment was studied at a time. Germ tube lengths were measured at 125 × every hour for 6 h for each temperature, by taking out the slides for measurement and then returning them to the humidity chambers. In each treatment, lengths of 30 germtubes (6-8 germtubes in each of the four replications) were measured. The experiment was done twice.

Effect of preinoculation storage temperature on the infectivity of sporangia. Samples (5 mL) of a sporangial suspension (1×10^5 sporangia mL^{-1}) were placed in 50 mm petri plates. A set of 12 plates was incubated at each of the eight temperatures: 10, 15, 20, 25, 30, 35, 40, and 45 C. A plate from each temperature was removed every hour for 12 h. Immediately after removal, 40-h-old seedlings grown at 30°C were immersed in each plate. Seedling age was kept constant for all inoculations by staggered plantings. After 2 h inoculation-incubation, at 20 ± 2°C, seedlings were transplanted into a sterile Alfisol soil (sterilized at 20 psi for 2 h a day for 3 days) in plastic pots (5 pots/treatment, each with 6-9 seedlings), which were maintained at 25-30°C in a greenhouse. Seedlings immersed in water for 12 h at each temperature served as checks. DM incidence was recorded 8 and 15 days after transplanting. The experiment was done twice.

Effect of postinoculation temperature on infectivity and disease development. Forty-hour-old seedlings grown at 30°C were immersed in a sporangial suspension (1×10^5 sporangia mL^{-1}) at 20°C in darkness. After 2 h, seedlings were transplanted into a sterilized Alfisol soil in pots. A set of five pots (each with 10 seedlings) was incubated at each of seven temperatures: 15, 20, 25, 30, 35, 40, and 45°C in a 12-h-dark/12-h-light cycle for disease expression. Seedlings similarly treated with water were maintained as checks for each treatment. DM incidence was recorded eight times between 3 and 13 days after treatment. The experiment was done twice.

Effects of light during inoculation on infectivity. Forty-hour-old seedlings were immersed in a sporangial suspension (1×10^5 sporangia mL^{-1}), in petri plates. A plate was incubated at 22°C in each of the four light regimes: (i) continuous light, (ii) continuous darkness, (iii) 12 h light followed by 12 h darkness, and (iv) 12 h darkness followed by 12 h light. Seedlings that were immersed in water for 24 h in light and dark separately were maintained as checks. After inoculation-incubation, seedlings from each treatment were transplanted into an Alfisol soil in five pots and maintained in the greenhouse. DM incidence records were taken 9 and 15 days after transplanting.

Effect of postinoculation light on disease development. Forty-eight-hour-old seedlings were immersed in a sporangial suspension (1×10^5 sporangia mL^{-1}) in a petri plate, which was incubated at 22°C for 2 h in darkness. The seedlings were then transplanted into an Alfisol soil in pots (15 seedlings per pot) under fluorescent light at 25 ± 2°C in a laboratory. A set of four pots was kept for disease development at 30°C in Percival incubators in each of three light regimes: (i) continuous light; (ii) continuous darkness, and (iii) 12 h light followed by 12 h darkness. Seedlings immersed in water were maintained as checks for each of the three treatments. Observations on DM incidence began 3 days after transplanting and continued daily or on alternate days until 22 days after transplanting. The experiment was done twice.

Relative susceptibility of roots and shoots to sporangial infection. *Root inoculation*—Water agar (5-mm thick) was poured into petri plates which were kept on a polythene sheet. Each plate had 50 equidistant holes (1-mm diam) in its bottom. After the agar had solidified, a seed was placed in the agar over each hole and the plate was placed above another plate full of water. Four such plates were incubated at 25°C in a 12-h-light/12-h-dark cycle. After 48 h, when roots had grown into the water in the lower plates, the water was replaced by a sporangial suspension (1×10^5 sporangia mL^{-1}), immersing the roots. After 2 h incubation at 20°C in darkness, seedlings from each plate were transplanted into an Alfisol soil in separate pots, which were maintained in a greenhouse for disease development. Roots immersed in water served as a check. DM incidence records were taken every day for 15 days. The experiment was done twice.

Shoot inoculation—Petri plates (90-mm diam) were filled to the brim with 1% water agar. Fifty surface-sterilized seeds were sown equidistantly in each plate. Four plates were incubated at 25°C in a 12 h light/12 h dark cycle. Roots grew down into the agar, and shoots grew upright. Forty-eight hours later, the plates were inverted to rest on

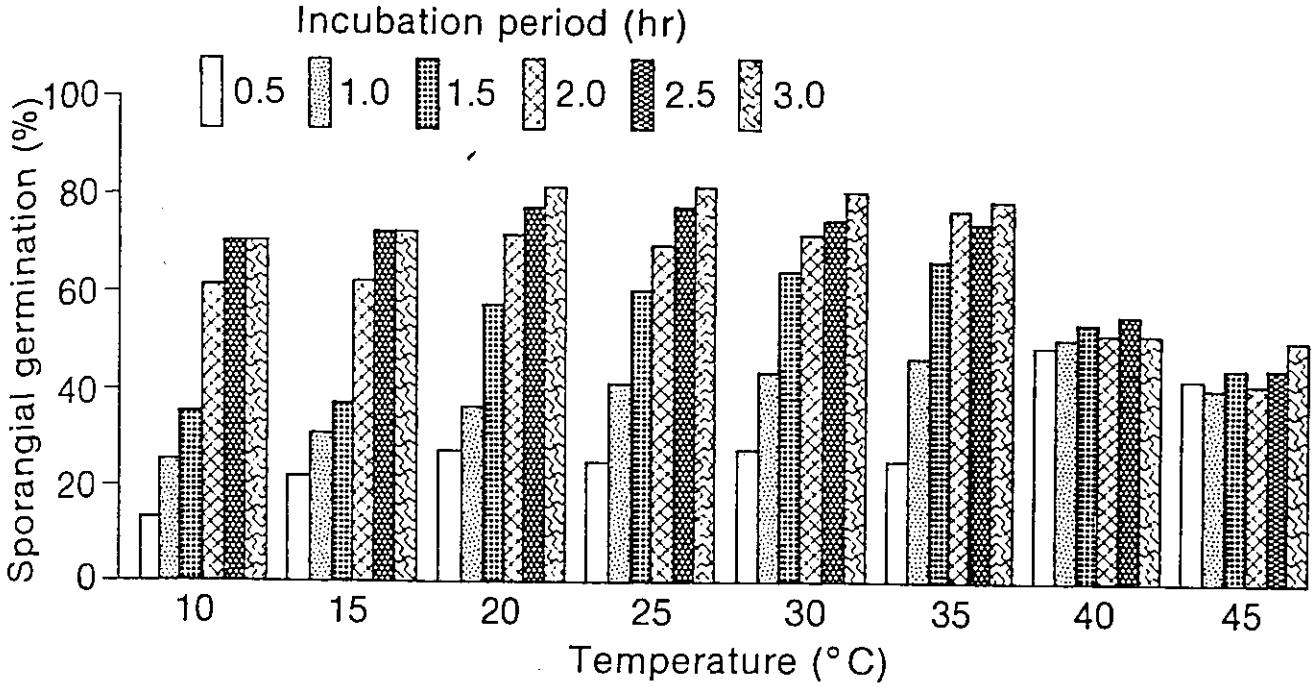

Figure 1. Effects of temperature and incubation period on germination of sporangia of *Sclerospora graminicola*.

similar plates filled with a sporangial suspension, thus immersing only the shoots in the inoculum. Four such plates were incubated at 20°C for 2 h in darkness, after which the seedlings were transplanted and maintained as above. Shoots immersed in water served as a check. DM incidence records were taken every day for 15 days. The experiment was done twice.

Combined root and shoot inoculation—Roots were inoculated following the procedure described for root inoculation. Shoots were inoculated by filling the space above the agar layer with sporangial suspension, immersing the shoots. Other details were as above. Roots and shoots immersed in water served as a check.

Results

Germination. Sporangia germinated (zoospore release) at all temperatures tested (Fig. 1). There was a gradual increase in germination with increase in incubation time from 30 min to 2 h at temperatures from 10 to 35°C. Germination was most rapid at 40 and 45°C, but total germination at these temperatures did not increase more than 60% even after 3 h. Regression analysis revealed that the optimum temperature for germination was 29.6°C ($R^2 = 0.88$); and little germination occurred after 2 h 40 min.

Germ tube growth. Zoospore germination and germ tube growth occurred at all the temperatures tested (Fig. 2). Germ tube length increased with increase in incubation time and temperature. The least growth occurred at 15°C and the most at 30°C.

Regression analysis was computed on the log-transformed values of germ tube lengths with time

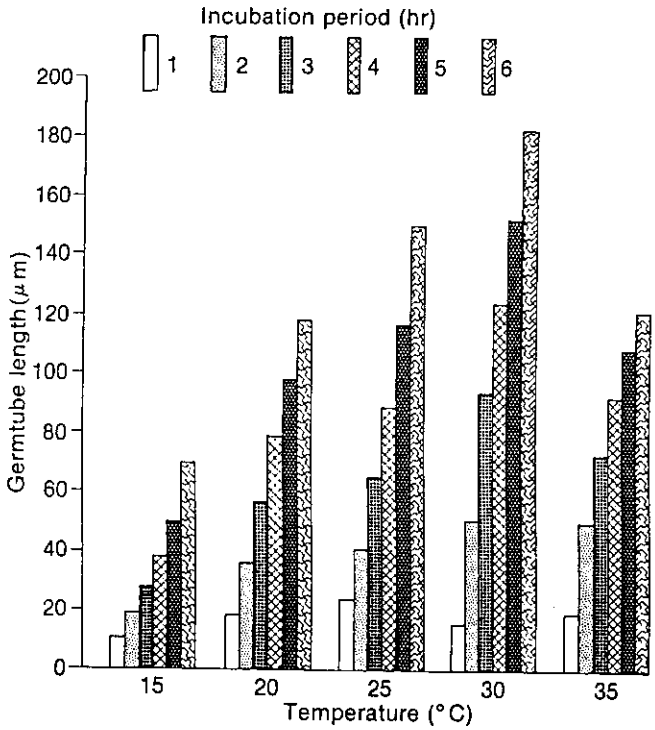

Figure 2. Effects of temperature and incubation period on the germ tube length of zoospores of *Sclerospora graminicola*.

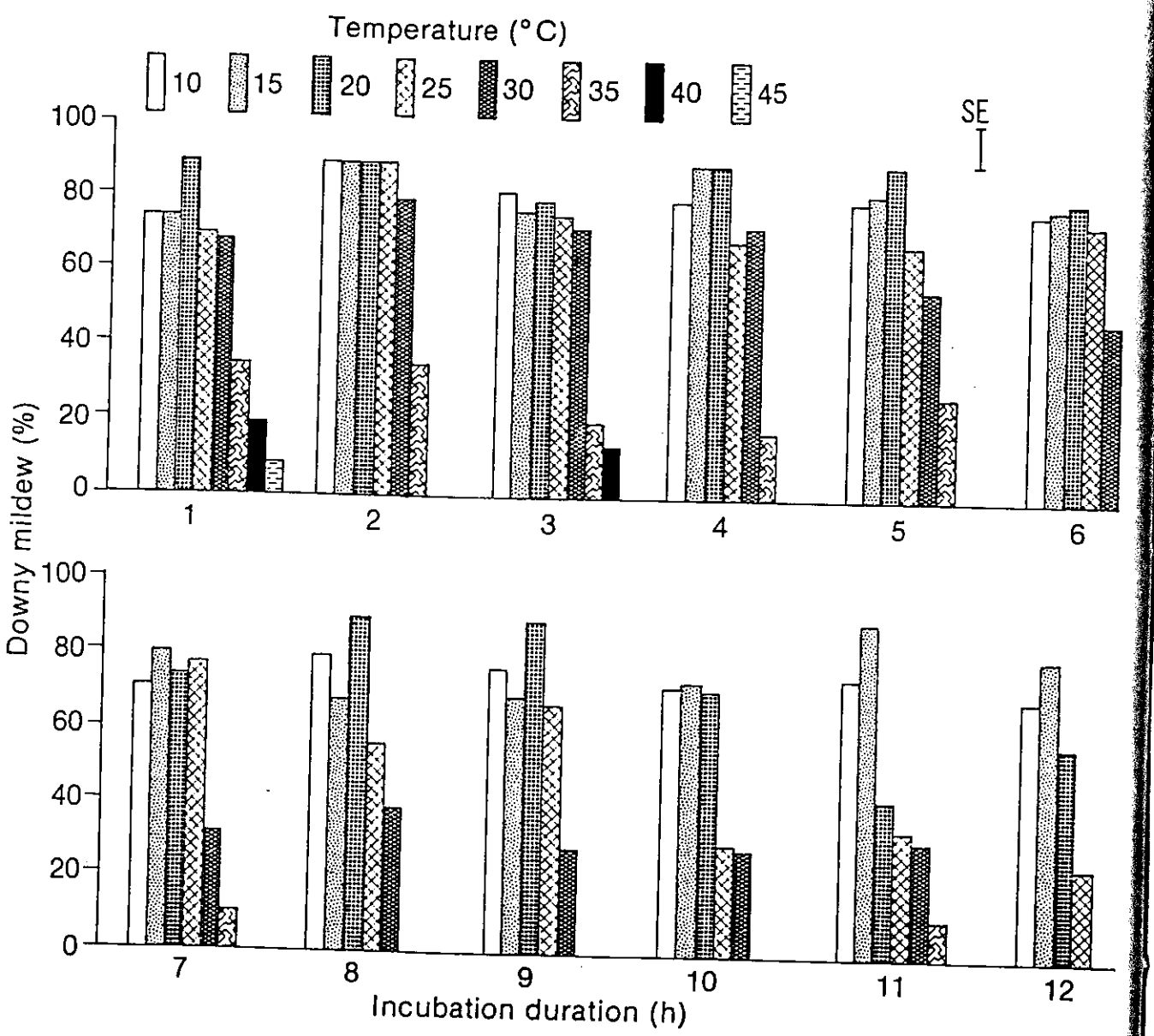

Figure 3. Effect of preinoculation storage temperatures on the infectivity of sporangia of *Sclerospora graminicola*.

at different temperatures and tests. The linear association adequately represented the growth relation of germ tube lengths with time. Intercept and slope values were analyzed for their differences across temperatures. The linear effect of temperature on intercept was highly significant ($P = 0.01$) and deviation from linearity was insignificant. This showed that temperature has no influence on the slope of germ tube length over time, since the growth lines at each temperature were parallel.

Effects of preinoculation storage temperature on infectivity. Infectivity of sporangia (zoospores), as judged by systemic infection of young seedlings, was greatly influenced by preinoculation temperature of the sporangial suspension. Sporangia stored at 10 and 15°C for up to 12 h produced ≥ 70% DM when used as inoculum (Fig. 3). Similar levels of DM also were recorded at 20, 25, and 30°C for up to 10, 7, and 4 h, respectively. Sporangial infectivity generally declined more rapidly at the higher incubation temperatures, with no infectivity after as little as 2 h incubation at the two highest temperatures, 40 and 45°C. None of the check seedlings developed DM.

Effect of postinoculation temperature on infectivity and disease development. Development of DM varied greatly with postinoculation temperature (Fig. 4). Seedlings incubated at 30°C developed DM symptoms as early as 3 days after inoculation. At 4 to 5 days after inoculation,

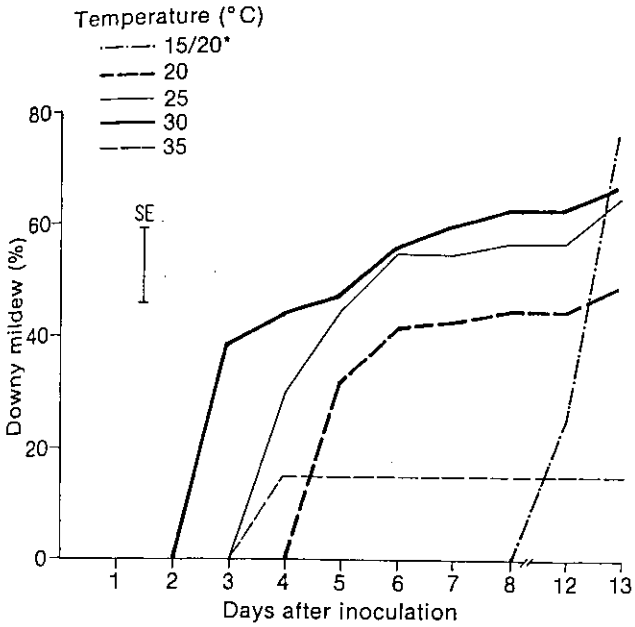

Figure 4. Development of downy mildew on sporangial inoculated (dip) transplanted seedlings of pearl millet cultivar 7042 incubated at several temperatures.
*After eight days incubation at 15°C, seedlings were moved to 20°C.

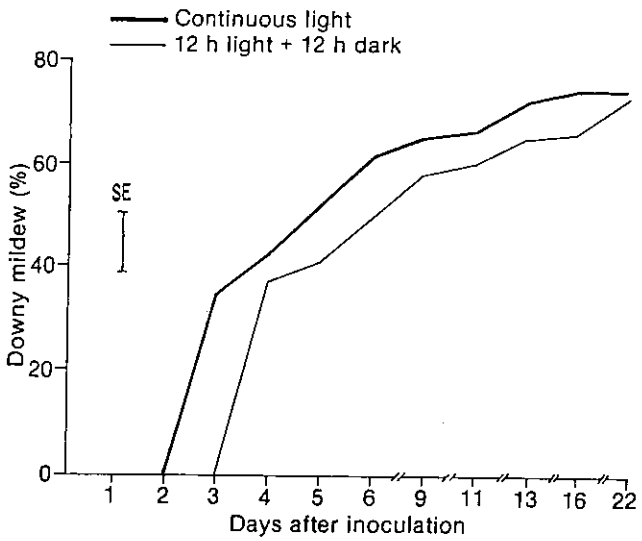

Figure 5. Effect of light and dark on downy mildew development.

seedlings at 20, 25, and 35°C also developed DM symptoms. Seedlings at 15°C remained symptomless for 8 days, but when they were then moved to 20°C, they developed > 75% DM within 5 days. DM did not develop at 40 and 45°C during up to 13 days after inoculation. None of the check seedlings developed DM.

Effect of light during inoculation on infectivity. A high DM incidence (≥ 86%) developed regardless of whether seedlings were exposed to the pathogen in the light or in darkness (Table 1). None of the check seedlings developed DM.

Effect of postinoculation light on disease development. DM symptoms appeared 3 days after inoculation in the continuous light treatment, and on the 4th day in the 12-h-light/12-h-dark cycle treatment. In both of these treatments, DM incidence increased sharply with increase in time (Fig. 5). No DM developed on seedlings in the 24-h dark treatment or in the check.

Relative susceptibility of roots and shoots to sporangial infection. Greater DM incidence occurred with shoot inoculation than by root inoculation (Table 2); however, combined inoculation of roots and shoots produced higher DM than did inoculation of roots or shoots alone.

Discussion

Survival and infectivity of sporangia are influenced by environmental factors, particularly temperature and humidity. Therefore, the potential of these units to cause epidemics is directly dependent on these environmental factors. The processes that occur during the period between sporangial production and infection include

Table 1. Downy mildew incidence (%) on seedlings of cultivar 7042 inoculated by immersion in a sporangial suspension of *Sclerospora graminicola* in light and/or dark

Treatment	No. of plants	% downy mildew	SE
Immersion in inoculum			
24 h in light	73	88.0	± 3.8
24 h in dark	87	86.0	± 3.7
12 h light + 12 h dark	66	92.0	± 3.3
12 h dark + 12 h light	76	88.0	± 3.7
Immersion in water			
24 h light	96	0	
24 h dark	94	0	

Table 2. Relative susceptibility of roots and shoots of 40-h-old pearl millet seedlings of cultivar 7042 to infection by sporangia of *Sclerospora graminicola*

Treatment	No. of plants	% downy mildew	SE
Immersion in inoculum			
Shoots only	188	68.3*	± 2.4
Roots only	173	38.8*	± 3.0
Roots & shoots	103	79.6	± 3.9
Immersion in water			
Shoots only	101	0*	
Roots only	113	0*	
Roots & shoots	90	0	

*Mean of two tests

dissemination of sporangia, zoospore release (sporangial germination), zoospore germination, germ tube growth, and penetration. The results from the present study show that sporangial germination and germ tube growth can occur under a wide range of temperatures, 10-45°C and 15-35°C, respectively. However, high levels of DM developed only between 20 and 30°C. The absence of disease at 15°C and low levels of DM at 35°C show that either penetration or the processes that follow penetration, are adversely affected at these temperatures. These results identify the environmental parameters that directly affect disease development in the field. Under field conditions, the processes from sporulation to penetration occur between midnight and sunrise. Temperatures during this period generally are $< 30°C$ during the cropping season and, therefore, are not a limiting factor for any of the processes. The RH, on the other hand, may be a limiting factor.

Low RH may inhibit sporulation (5) or the processes following sporulation, eg. germination and germ tube growth. Thus, in the absence of high RH provided by rainfall and/or irrigation in the early seedling stage of the crop, a susceptible cultivar may behave as resistant under field conditions. The results of the study on the effect of light on germination and infectivity of sporangia may not be of much practical significance because under natural conditions these processes occur at night. The results presented here indicate that the pathogen is highly versatile and remains unaffected by considerable change in environmental factors, particularly temperature.

The effect of temperature on germination of sporangia has been reported by several workers (2). Our results agree with those of others (1,3,9,10) in that sporangial germination begins after about 30 min incubation. However, our results differ in relation to the effect of temperature. We found that sporangia germinated even at 45°C, whereas the highest temperature reported earlier for germination was 33°C (1).

Roots and shoots are both susceptible to infection by zoospores. The high levels of DM developed by the combined inoculation of both roots and shoots, however, show that for developing high levels of infection, minimizing escapes, and detecting true resistance, both roots and shoots should be exposed to the inoculum, as has already been emphasized (6). Thus field screening for DM resistance should be done in oospore-infested plots (plots containing unknown numbers of oospores), with sporangial inoculum supplied by infector rows. Sporangia incubated at 10 to 30°C can survive for 4-12 h and cause infection, provided they remain in water. In the absence of water, they collapse within a few minutes, irrespective of the temperature of storage (R. Gopinath, and S.D. Singh, unpublished). However, their abundant production for several days in infected leaves, their rapid germination, and the short period required for penetration and incubation, all occurring over a wide range of temperatures, largely outweigh their short-lived nature and give them an important place in the epidemiology of this disease, which is widespread and destructive throughout the subtropics.

The authors thank S.B. King for his constructive criticism of the manuscript and gratefully acknowledge the valuable guidance of Murari Singh with statistical analyses.

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