Effects of Dew-Period Temperature on Germination of Conidia and Systemic Infection of Maize by Sclerospora sorghi

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ABSTRACT

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The optimum temperatures for conidia germination and germ-tube growth of an American isolate of *Sclerospora sorghi* were 15 and 22 C, respectively. Germination, however, was high from 10 to 19 C and germ-tube growth was good from 14 to 22 C. An air temperature range of 14 to 22 C during a 2-hr dew period was near optimum for systemic infection of maize. Systemic infection consistently occurred from 10 to 33 C when plants were exposed to a dew-chamber period of at least 4 hr. The American isolate had a lower optimum temperature range for conidia germination than that reported for an Indian isolate of *S. sorghi* which indicates the existence of biotypes.

Additional key words: epidemiology, sorghum downy mildew.

Sorghum downy mildew, which is incited by *Sclerospora sorghi* Weston and Uppal, is a serious disease of both sorghum and maize. This and related downy mildews of maize are of major importance in tropical and subtropical areas of the world. Although sorghum downy mildew has been known in Asia and Africa for several decades, it was not reported to occur in the United States until 1961, when the disease was observed at College Station and Chillicothe, Texas (4). Since 1961, *Sclerospora sorghi* has spread as far north as southern Indiana (8). The discovery of this pathogen in Texas and its rapid movement northward to the corn belt demand our serious attention and concern. There is a paucity of information on epidemiology of the disease, especially for collections of the pathogen from the United States.

The purposes of this research were to study the effects of temperature on germination of conidia and germ-tube growth of an American isolate of *S. sorghi*, and to study the effect of dew-period temperatures on systemic infection of maize. We concentrated on systemic infection as a measure of disease because of ease of recognition and because the systemic infection phase of the disease results in most of the yield loss caused by sorghum downy mildew (4).

MATERIALS AND METHODS

A culture of *S. sorghi*, obtained from R. A. Frederiksen in Texas in 1972, was maintained in the greenhouse on maize and sorghum. In all experiments, freshly harvested conidia were used on inocula and studies were conducted in a quarantine containment facility.

Preparation of inoculum.—Conidia were collected from infected "donor" plants of *Sorghum bicolor* (L.) Moench 'TX412' or *Zea mays* L. 'DeKalb XL-43' previously inoculated and then maintained in the greenhouse for 3 to 5 wk. Prior to spore collection, the donors were exposed to supplemental light (from Sylvania 1,000-W Metalarc high-intensity lamps) for approximately 12 hr (2000-0800 hours) and then placed in a dark dew chamber (5) at about 19 C for 5.5 hr to induce sporulation. Conidia were collected by washing the spores from donor leaves with a fine stream of cold (about 5 C) distilled water delivered by an atomizer at about 3,500 Kg/m^2 (~5 lb/in^2) air-line pressure. The spore suspension was filtered immediately through a 44-μm (325-mesh) screen, the initial spore concentration determined by means of a hemactometer, and the spore suspension was adjusted to the desired concentration by dilution with cold distilled water.

Systemic infection studies.—Four separate experiments were conducted to determine the relationship of air temperature during dew periods and subsequent frequency of systemic infection. Near-constant air temperatures in dew chambers ranged from approximately 8 to 33 C. In two of the experiments, XL-43 maize seedlings were inoculated with a spore suspension (8×10^4 spores/ml) obtained from TX412 sorghum donor plants. In the other two experiments, infected XL-43 maize served as the donor and inoculum was prepared at 2.5 or 3.7×10^4 spores/ml of spore suspension.

Seven- to 8-day-old XL-43 maize seedlings were inoculated while in dew chambers by spraying the seedlings (two plants/10.2-cm diameter clay pot) with the

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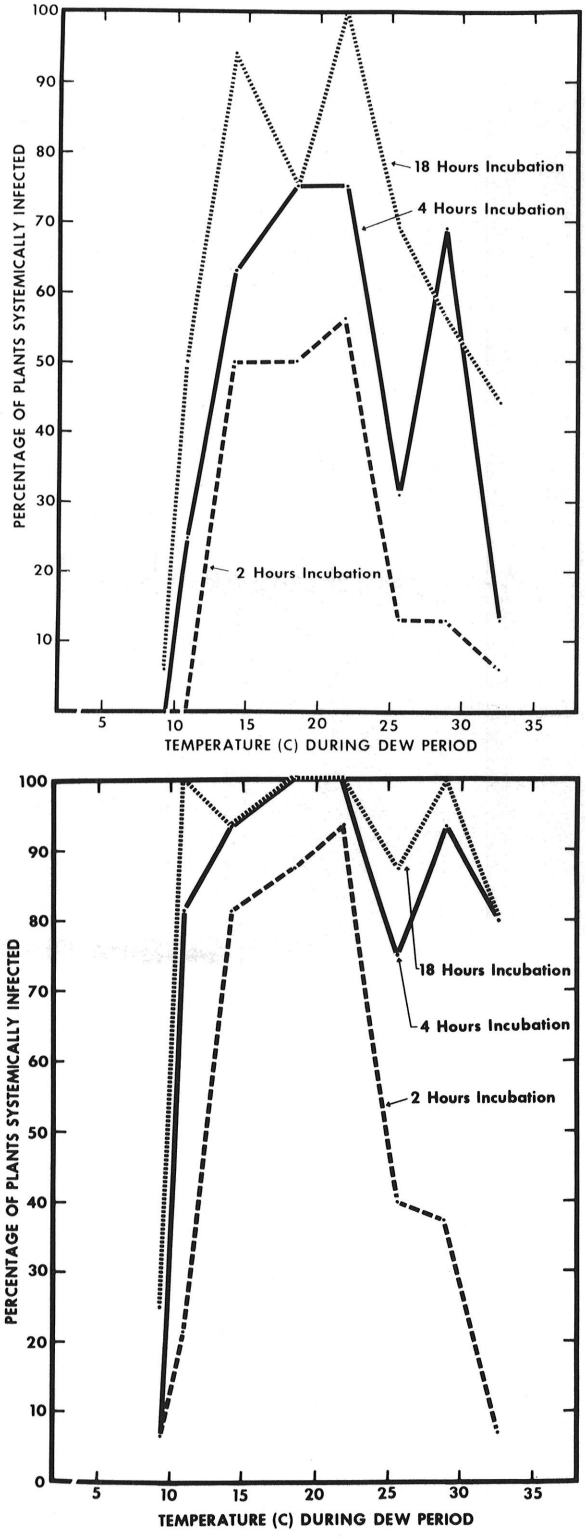
spore suspensions (0.5 ml/plant). The inoculated plants were maintained in the dew chambers, previously equilibrated at the desired air temperatures, for 2, 4, or 18 hr. Sixteen plants were used for each temperature-dew period combination in each experiment. The air temperature in each chamber was monitored continuously by a thermocouple during the dew periods. Following the dew periods, inoculated and controls plants were placed in the greenhouse for disease development. The normal temperature fluctuation in the greenhouse was 21 to 28 C; however, on occasion it peaked as high as 40 C shortly after noon.



Conidial germination and germ - tube growth studies.—To determine the effect of specific temperatures on conidia germination and germ-tube growth, a conidial suspension was sprayed onto 1.25% water agar in small plastic petri plates (35 × 10 mm). Two or three replicate plates were used, depending on the particular experiment. The spore density on the agar surface averaged 1 to 5 spores/mm^2. Agar-plate temperatures were equilibrated with the chamber air temperatures prior to seeding the plates. During incubation, petri plate tops were covered with aluminum foil and lined with filter paper to prevent condensation water from falling onto the

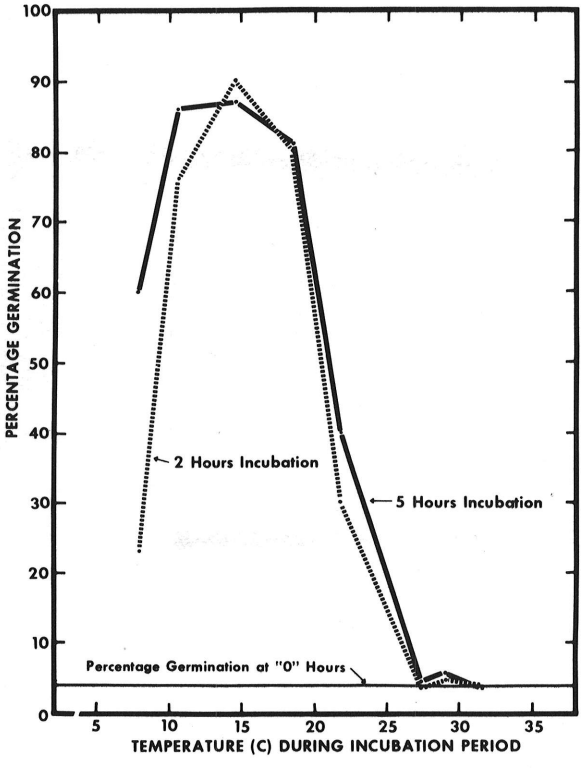


Fig. 3. The percentage of conidia which germinated on 1.25% water agar during incubation at specified temperatures for 2 or 5 hr in the dark. Each point represents the mean percentage germination of 100 conidia in each of three petri plates.

Fig. 1-2. The percentage of XL-43 maize plants with systemic infection **1)** 14 days or **2)** 24 days after inoculation with a syspension (8.0 × 10^4 spores/ml) of *Sclerospora sorghi* conidia. After inoculation, the seedlings were incubated in the dark in dew chambers at the specified air temperatures for 2, 4, or 18 hr before being placed in a greenhouse at 21 to 28 C. Each point represents 16 plants.

agar surface. Air temperatures within the chambers were monitored throughout incubation periods. For a representative experiment [except for a temporary ($\leq$ 10 min) change of $\pm$ 4.0 C from the mean air temperature that occurred when the chamber door was opened during seeding of the plates] the chambers were within $\pm$ 1.2 C of the stated mean temperatures.

At the end of the incubation period, the plates were opened and placed over 38% formaldehyde in a desiccator to immediately kill the spores. Germination percentages were determined by microscopic observation of at least 100 spores per plate at $\times$100 magnification. A spore was considered germinated if the length of the longest germ tube was equal to or greater than the width of the spore. The longest germ tube of each spore of 20 randomly selected germinated spores per petri plate was measured at $\times$100 magnification to determine mean length of germ tubes at the different temperatures.

Three experiments were performed to determine the optimum temperatures for conidiospore germination and germ-tube growth; one experiment involved determination of germination percentages following 2, 4, or 18 hr of incubation at various temperatures from 9 to 33 C and in the other two experiments germination percentages and germ-tube lengths were determined following 2 or 5 hr of incubation at selected temperatures from 8 to 32 C.

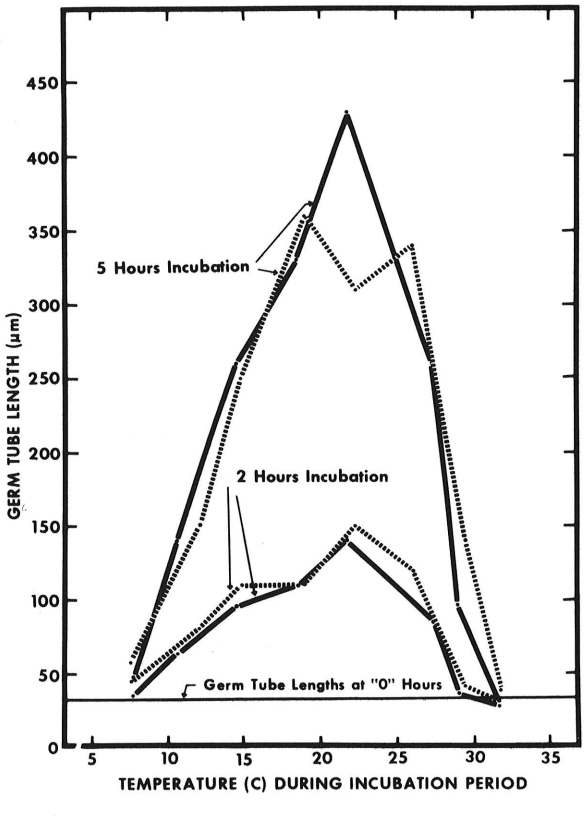


Fig. 4. Mean germ tube lengths (μm) after 2 or 5 hr of incubation on 1.25% water agar in the dark at the specified temperatures. Each point represents the mean length of the longest germ tube for each spore of 20 randomly selected germinated conidia in each of three petri plates. The solid lines represent data from one experiment and the dotted lines data from a second experiment.

RESULTS

Systemic infection studies.—In three of the four temperature experiments, high levels of systemic infection occurred from 11 to 32 C and temperature during the dew-chamber period was not critical in determining the prevalence of systemic infection. For instance, in one experiment with an 18-hr dew period, 96-100% of the inoculated plants at each temperature from 11 to 32 C became systemically infected, and at 8 C, 71% of the plants developed systemic infection. In another experiment, 94-100% of the inoculated plants were systemically infected at all temperature and dew length combinations except with the two lowest temperatures (9.7 or 11.4 C) in combination with the dew length of shortest duration (2 hr).

In one experiment, however, there was an apparent near-optimum temperature range of 14 to 22 C (with 2- or 18-hr dew periods) for systemic infection when plants were examined 14 days after inoculation (Fig. 1). However, the optimum temperature range for the 4-hr dew treatment was different; there was a higher incidence of disease at 29 C than might be expected when comparing results of the 4-hr dew treatment with numbers of plants developing systemic infection at 29 C under the 2-hr or 18-hr dew treatments. After 24 days, the number of plants systemically infected had increased (Fig. 2) and a relatively narrow near-optimum temperature range was apparent with only the 2-hr dew-chamber period. With the 4- or 18-hr periods, most plants were systemically infected at all temperatures from 11 to 33 C, the maximum temperature tested.

Studies of conidia germination and germ-tube growth.—In all three experiments the optimum temperature for germination was 15 C. Germination was nearly as high at 10 and 19 C as at the optimum, however germination always was much reduced at 22 C (Fig. 3).

The optimum temperature for germ-tube growth was 22 C, but growth was good from 14 to 27 C (Fig. 4).

DISCUSSION

There are few reports on the optimum temperatures for conidia germination of the downy mildew pathogens that infect maize. *Sclerospora sacchari*, a pathogen of both sugarcane and maize, has been reported to have an optimum range of 19 to 28 C (2), and *S. philippinensis* has a reported optimum range of 19 to 24 C (3). Safeeulla et al. (6) determined the optimum temperature for conidia germination of *S. sorghi* in India to be 21 to 25 C. We obtained results nearly identical to those of Safeeulla et al. with tests of conidia of an isolate of *S. sorghi* from Thailand (Bonde, *unpublished*). Although Schmitt and Freytag (7) previously reported that rapid germination of the Texas isolate of *S. sorghi* occurred from 13 to 27 C, our results from more precisely controlled experiments in this study demonstrated that the optimum temperature for conidia germination of this culture was 15 C with nearly as high germination from 10 to 19 C. Whereas germination at 10 C was near optimal for the Texas

culture, Safeeulla et al. obtained no germination at this temperature. In India, the researchers reported 80% germination at 32 C for *S. sorghi*, whereas we found little germination at 27 C or above with the Texas culture. Results obtained in India and in our study indicate the existence of biotypes of *S. sorghi* with markedly different optimum temperatures for germination. It is possible that the lower optimum for germination of the American isolate represents adaptation to the more temperate environment of the continental USA. We presently are comparing the isolate of *S. sorghi* from Thailand with the pathogen from Texas. Comparisons under identical conditions should be made among isolates of *S. sorghi* from several parts of the world to determine the extent of variation in the environmental requirements of this species.

Germ-tube growth of the Texas isolate of *S. sorghi* is near maximum from 14 to 27 C, a temperature range somewhat higher than the near-optimum temperature range of 10 to 19 C for germination. Although, in most instances, there was little difference in the incidence of systemic infection with dew-period temperatures ranging from 11 to 33 C, in one experiment there was a narrower near-optimum of 14 to 22 C. This near-optimum temperature range for systemic infection includes the higher end (above 14 C) of the near-optimum temperature range for germination and the lower end (below 22 C) of the near-optimum for germ-tube growth. This suggests that conidia germination is the more limiting factor in initiation of disease above 14 C and that germ-tube growth is the more limiting factor below 14 C. This near-optimum temperature range of 14 to 22 C during dew for systemic infection was apparent in another experiment in which plants had been exposed to dew for 2 hr, the shortest duration tested, and examined for the presence of systemic infection at 14 days.

We believe that inoculation of plants at the relatively high spore concentrations that we used may have frequently overwhelmed the plants and allowed high amounts of systemic infection under sub-optimal conditions for disease. It is possible that inoculation of plants at a lower spore concentration, possibly 1×10^3 or 5×10^3 spores/ml, would have resulted in the narrower near-optimum temperature range for disease regardless of the duration of the dew period and might have been more representative of inoculum concentrations under field conditions. This aspect shall be tested in the future.

Percentage germination of conidia on water agar was very low at 27 and 32 C and germ tubes that formed at 32 C usually grew slowly. However, despite this poor germination and germ-tube growth, about 1% of the spores that produced germ tubes at 32 C had tubes about 180 μm in length after 5 hr. Spores of this type were probably responsible for systemic infection that occurred with the 32 C dew-period treatment.

With the present data we cannot conclusively say that all infection resulted from spores that germinated during the dew-chamber periods. When plants were removed from the chambers, moisture remained in leaf whorls for periods as long as several hours. It is probable that some conidia germinated after the prescribed 2- and 4-hr dew periods, and therefore germination and germ-tube growth may have occurred in moisture at temperatures more favorable than prevailing temperatures during the dew-chamber periods. However, with 18 hr of dew it is unlikely that any of the observed systemic infection resulted from penetration which occurred after the end of the dew-chamber period. The delicate conidia of *S. sorghi* are viable for only 3 to 4 hr (1) except when refrigerated (Schmitt and Bonde, *unpublished*).

In histological studies we have determined that conidia of *S. sorghi* under favorable conditions germinate rapidly and gain ingress into maize leaves within 2 hr. Since some moisture necessarily remains deep within leaf whorls for at least 2 hr following inoculation, a rare plant will sometimes become systemically infected without even being placed in dew. This very rapid conidia germination and penetration is necessary for a pathogen whose conidia are viable for only a few hours.

It is epidemiologically significant that systemic infection consistently occurred with high inoculum concentrations at 10 to 33 C when plants were exposed to dew periods of at least 4 hr.

LITERATURE CITED

1. ANONYMOUS. 1973. A compendium of corn diseases. American Phytopathological Society, St. Paul, Minnesota. 64 p.
2. CHANG, S. C., and T. H. WU. 1969. Effects of temperature and humidity on the production and germination of Sclerospora sacchari conidia on corn. Pages 125-130 *in* The Sixth Inter-Asian Corn Improvement Workshop, 26-30 September 1969, U. P. Agricultural University, Pantnagar (Nainital), India. 155 p.
3. EXCONDE, O. R., E. A. EDRALIN, and B. A. ADVINCULA. 1967. Some factors affecting formation and germination of spores of Sclerospora philippinensis. Pages 111-122 *in* The Fourth Inter-Asian Corn Improvement Workshop, 8-14 October 1967, Lyallpur, West Pakistan. Sponsored by Dept. of Agric., Govt. of West Pakistan. 144 p.
4. FREDERIKSEN, R. A., A. J. BOCKHOLT, L. E. CLARK, J. W. CASPER, J. CRAIG, J. W. JOHNSON, B. L. JONES, P. MATOCHA, F. R. MILLER, L. REYES, D. T. ROSENOW, D. TULEEN, and H. J. WALKER. 1973. Sorghum downy mildew: A disease of maize and sorghum. Tex. Agric. Exp. Stn. Tech. Monogr. 2. 32p.
5. LANG, C. T., C. H. KINGSOLVER, J. E. MITCHELL, and E. CHERRY. 1958. Determination of the effects of different temperatures on uredial infection with Puccinia graminis var. tritici. Phytopathology 48:658-660.
6. SAFEEULLA, K. M., H. S. SHETTY, and N. G. P. RAO. 1974. Sorghum downy mildew. 1973-1974 Res. Rep., Dept. of Post-Graduate Studies and Research in Botany, Manasagangothri, Mysore. 49 p.
7. SCHMITT, C. G., and R. E. FREYTAG. 1974. A quantitative technique for inoculating corn and sorghum with conidia of Sclerospora sorghi, Plant Dis. Rep. 58:825-829.
8. WARREN, H. L., D. H. SCOTT, and R. L. NICHOLSON. 1974. Occurrence of sorghum downy mildew of maize in Indiana. Plant Dis. Rep. 58:430-432.