Effects of Dew-Period Temperature on Sporulation, Germination of Conidia, and Systemic Infection of Maize by *Peronosclerospora sacchari*

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ABSTRACT

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Peronosclerospora sacchari sporulated profusely on maize from 15 to 23 C. At 26 C and above, conidiophores were malformed and produced few spores. Temperatures between 15 and 30 C were equally favorable for germination in vitro. With 5-hr incubation, in a representative test, germination was 58–86% between 8 and 35 C, the entire range tested. After 2 hr, spores incubated at 26 C had the longest germ tubes (average length 315 μm); germ tubes were long (>56% of maximum) from 18 to 32 C. The temperature range during the dew chamber period most favorable for infection and subsequent disease development was 18–32 C (63–81% infection for a representative experiment), but incidence of infection was nearly as high at 12 C (44–53%). At 8 C, the lowest temperature tested, 6% of the plants became systemically infected. After inoculation by atomizing with a conidial suspension, a 4-hr dew period was as effective as an 18-hr period in inducing systemic infection. Temperature and moisture conditions favorable for infection of maize by *P. sacchari* are common during the growing season in much of the United States corn belt.

Additional key words: Sclerospora sacchari, sugarcane downy mildew, epidemiology, corn.

Sugarcane downy mildew of maize, incited by *Peronosclerospora sacchari* Miyake, has never been reported in North America but has been a very serious disease of both sugarcane and maize in the Orient (10). In Taiwan from 1960-1964, 70% of the popular maize variety Tainan No. 5 grown in the Chiayi-Tainan area was affected (10). Since 1964 the disease has decreased markedly in Taiwan, primarily because of the development of resistant sugarcane and maize varieties. Maize varieties currently grown in the United States, however, are highly susceptible to *P. sacchari* (Bonde, *unpublished*), and American breeding lines that are highly resistant to *P. sorghi* in the United States are highly susceptible to *P. sacchari* (8; Bonde, *unpublished*).

The epidemiology of sugarcane downy mildew of maize has been studied in the Orient (2,4,6,7,9,10), but we believe further studies must be done to determine the threat of *P. sacchari* to the American maize crop should this pathogen enter and establish itself.

The purposes of this research were to study the effects of temperature on sporulation, germination of conidia, and growth of conidial germ tubes. Effects of dew-period temperature on systemic infection of maize also were studied. Systemic infection was used as a measure of disease because of ease of recognition and because the systemic infection phase of maize downy mildews causes most, if not all, yield loss from these diseases (3).

MATERIALS AND METHODS

Two cultures of *P. sacchari,* isolates I and 77B, were obtained from S. C. Chang in Taiwan in 1975 and 1977, respectively. Single conidiophores bearing spores were used to establish subcultures, and each subculture was maintained in the greenhouse on maize plants, *Zea mays* L. 'DeKalb XL-43.' In all experiments, freshly harvested conidia were used as inocula, and studies were conducted in a quarantine containment facility.

Preparation of inoculum. Conidia were collected from infected donor plants previously inoculated with either of the isolates and then maintained in the greenhouse for 3–5 wk. Before spore collection, the donors were exposed to supplemental light (Sylvania 1,000 W Metalarc high-intensity lamps) for about 15 hr (1600–0700 hours) and then placed in a dark dew chamber (5) at 21–23 C for 5–7.5 hr (depending on the isolate and experiment) to induce sporulation. Isolate I generally required about 2 hr more dew than isolate 77B. In the dew chambers, condensation formed on the surface of plants when their surface temperatures dropped below the dew point, thus simulating nature. 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 was determined with a hemacytometer, and the spore suspension was adjusted to the desired concentration by dilution with cold distilled water.

Systemic infection. In a series of experiments, the relationship of air temperature during dew periods after inoculation and subsequent frequency of systemic infection in inoculated plants was determined. DeKalb XL-43 or Pioneer 3369A seedlings in the first-leaf stage (two per 10-cm diameter clay pot) were placed in dew chambers at selected near-constant air temperatures ranging from 8 to 32 C. After temperature equilibration, the seedlings were sprayed with a spore suspension (5×10^3 spores per milliliter, 0.25 ml per plant) of isolate I or 77B and then were held in the chambers at the desired air temperatures for 4 or 18 hr. Sixteen plants were used for each temperature-dew period combination in each experiment. Noninoculated plants or plants sprayed with distilled water were incubated 4 or 18 hr at a favorable temperature for infection in another dew chamber and served as controls. During the dew period, the air temperature in each chamber was monitored continuously with a thermocouple and recorder. In a representative experiment, the air temperatures were always within ± 1.4 C of the stated means except for a temporary (<13 min) change (from the normal fluctuation) when the chamber door was opened during inoculation. This temporary change was <2 C at all temperatures except the highest for which the change was a maximum of 6 C and

lasted 13 min. Plants were examined routinely for dew deposition 4 hr after inoculation.

After the dew periods, inoculated and control seedlings were placed in the greenhouse. All plants were examined for symptoms of systemic infection 24 days after inoculation. Air temperature in the greenhouse fluctuated from 21 to 27 C.

Conidiospore germination and germ-tube growth. In tests on the effect of specific temperatures on conidiospore germination and germ-tube growth, a conidial suspension was sprayed onto 1.25% water agar in plastic petri plates (48 × 8 mm). In each experiment three replicate plates were used at each temperature for each incubation period; the spore density on the agar surface was two to seven spores per square millimeter. Agar-plate temperatures were equilibrated with the chamber air temperatures before the plates were seeded. During incubation, petri plate tops were covered with aluminum foil and were lined with filter paper to prevent condensed water from falling onto the agar surface. Air temperatures in the respective chambers were monitored throughout incubation with thermocouples connected to a 12-point recorder. Prior tests with calibrated mercury-bulb thermometers showed that the temperature of the agar surface was always within ± 1.2 C of the stated mean temperature, even when the chamber door was open during seeding of plates.

After incubation, the plates were opened and placed over 38% formaldehyde in a desiccator jar to kill the spores quickly. Germination percentages were determined by microscopic observation of 100 spores per plate. A spore was considered germinated if the length of the longest germ tube exceeded the width of the spore. The germ tube of each of 20 randomly selected germinated spores per petri plate was measured at ×150 magnification to determine mean length of germ tubes at the different temperatures. If a spore had more than one germ tube, only the longest was measured.

Sporulation. In three experiments with isolate *P. sacchari* I and one experiment with isolate 77B, the relationship was determined between temperature during the dew period and development of conidiophores and conidia on systemically infected maize plants. Infected XL-43 plants (3–4 wk after inoculation) were incubated in dew chambers for up to 7.5 hr; between 5 and 7.5 hr, leaf pieces were excised at 0.5-hr intervals from the plants and immediately fixed in absolute ethanol/acetic acid (2:1, v/v). After at least 12 hr in the fixative, leaf pieces were stained with 0.1% cotton blue in lactophenol and observed microscopically for development of conidiophores and conidia.

RESULTS AND DISCUSSION

Systemic infection. Temperatures between 18 and 32 C during the dew period, regardless of duration, were equally favorable for subsequent development of systemic infection; however, incidence of disease was nearly as great at 12 C, and a few plants became systemically infected at 8 C, the lowest temperature tested. Results of an experiment representative of either isolate are presented (Fig. 1). The high temperature of 32 C apparently did not inhibit infection (Fig. 1). In a few experiments, 100% of the plants became infected between 18 and 32 C. The maximum infection obtained at 8 C was 25%. Numbers of plants developing systemic infection differed little between 4- and 18- hr dew periods.

Conidiospore germination and germ-tube growth. With either isolate, temperatures for germination were favorable from 15 to 32 C. Germination usually was high from about 12 to 32 C with a 2-hr incubation and from 8 to 35 C with a 5-hr incubation (Fig. 2). In one experiment in which 6 C was the lowest temperature tested, spores did not germinate at this extreme even after 5 hr.

Optimum temperature for germ-tube growth of either isolate was 26 C, and growth was extensive from 20 to 30 C (Fig. 3).

The most favorable temperature range for germination was 19–28 C as reported by Chang and Wu (2), with an optimum about 25 C, as reported by Sun (9). Leu and Tan (7) obtained 100% germination of conidia of *P. sacchari* in 80 min from 8 to 32 C, the entire range they tested. The latter workers did not report whether they allowed the germination medium to equilibrate with the test

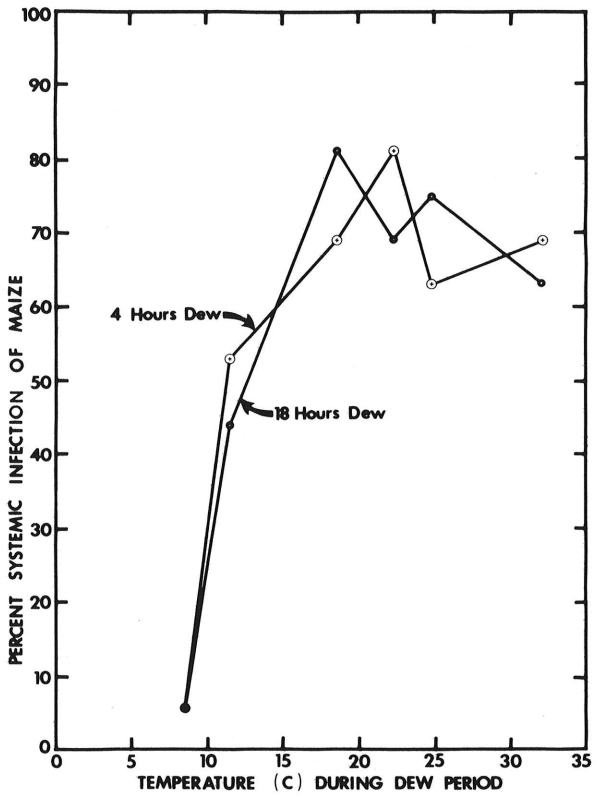

Fig. 1. Percent of maize plants with symptoms of systemic infection after inoculation with *Peronosclerospora sacchari* isolate I conidia (5×10^3 spores per milliliter, 0.25 ml per plant), incubation of plants 4 or 18 hr in dew chambers over a temperature range, and placement of plants in a greenhouse for 24 days.

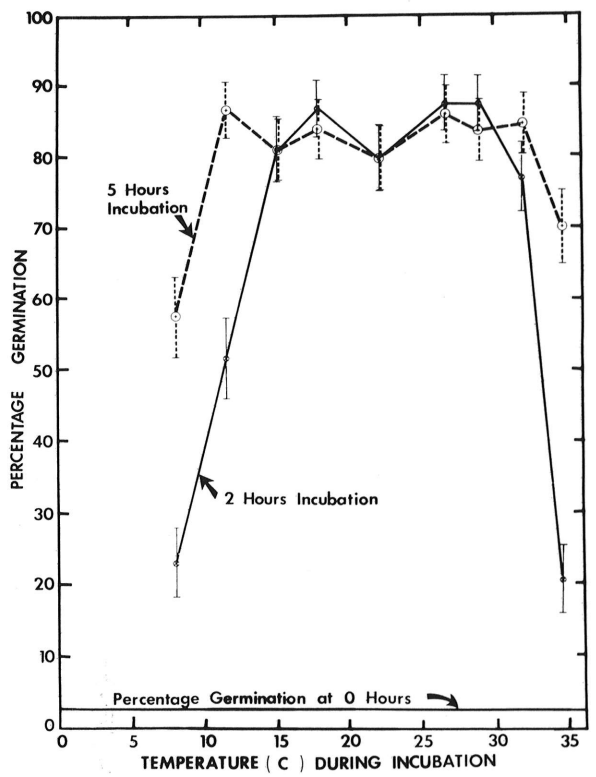

Fig. 2. Germination percentages from a representative experiment with *Peronosclerospora sacchari* isolate I conidia after 2 or 5 hr incubation on 1.25% water agar at specified temperatures. Confidence limits (95%) indicated.

temperature before seeding with spores, which could influence the interpretation of their temperature-response curve. Nevertheless, *P. sacchari* germinates exceptionally well over a very broad temperature range. An isolate of *P. sorghi* from Texas germinated well over a much narrower temperature range and had a lower optimum for germ-tube growth (1).

Sporulation. Each isolate sporulated profusely from 15 to 23 C. At 26 C and above, conidiophores were malformed and produced few spores. Isolate I reached its estimated peak of sporulation in 6–7 hr at 18 to 23 C, whereas isolate 77B generally required only 5–6 hr at an optimum temperature. An additional hour with dew was required at 15 C for each isolate.

Leu (6) determined that the most favorable range for sporulation was 22–26 C. Sun (9) reported that the optimum temperature for sporulation in Taiwan was about 25 C and that, with night temperatures of 20–25 C and free moisture on the leaf surface, millions of conidia were produced on a single infected plant.

Other considerations. With many fungal pathogens of plants, teperature during the dew period affects sporulation, spore germination, germ-tube growth, and eventual establishment of infection. Knowledge of the specific environmental requirements of *P. sacchari* during this critical period should help to determine the threat to the American maize crop if *P. sacchari* enters the United States and becomes established. Likewise, information on temperature and moisture conditions in maize growing areas of the United States during the first month of plant growth is required. Plants are most susceptible to infection immediately after seed germination and emergence; after a month, they become nearly immune to subsequent infection (9).

At the Plant Disease Research Laboratory (Frederick, MD) air temperatures, leaf temperatures, and free moisture on the surface of plants have been monitored in recent years. Beginning about June 1, temperature and moisture conditions on most evenings in Frederick are favorable for sporulation and infection of maize by *P. sacchari*. Comparable information, expecially at the leaf surface, is not readily available from other locations. We believe, however, that moisture and temperature conditions in the north central area of Maryland are typical of many United States maize-growing areas.

Even if it enters the United States, *P. sacchari* probably would not threaten maize production unless a source of inoculum was near maize fields early in the development of the plants. Investigations need to be conducted to determine if other potential hosts that would provide initial conidial and/or oospore inoculum exist in the United States and to determine if oospores that might overwinter and initiate infection the next spring are produced in American maize varieties.

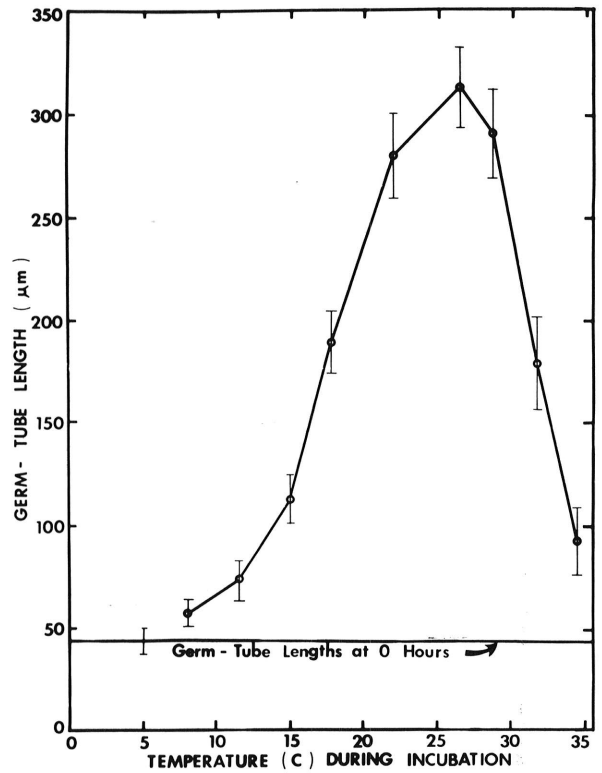

Fig. 3. Germ-tube lengths from a representative experiment with *Peronosclerospora sacchari* isolate I after 2-hr incubation of conidia on 1.25% water agar at specified temperatures. Confidence limits (95%) indicated.

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