Tiered Temperature System for Producing and Storing Conidia of *Peronosclerospora sorghi*

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ABSTRACT

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A system was devised for producing and preserving conidia of *Peronosclerospora sorghi* for use as inoculum. The procedure used natural forces to transfer mature conidia produced on diseased sorghum leaves to water agar chilled to temperatures that inhibited conidial germination. Conidia produced by production-storage intervals (time interval between incubation of diseased leaves and collection of conidia from agar surface) of 16 and 24 hr and agar temperatures of 3 and 5 C germinated after collection at frequencies ranging from 81 to 92% with no significant differences between storage intervals or temperatures. Germination of conidia produced by a 32-hr production-storage interval was less, ranging from 41 to 57%. Conidial inoculum produced by a 24-hr production-storage interval induced a 99% incidence of downy mildew in susceptible corn seedlings. The system permits greater flexibility in the timing of inoculum production and inoculation than older methods.

Peronosclerospora sorghi (Weston & Uppal) Shaw, the causal agent of sorghum downy mildew, caused severe losses in sorghum production in Texas in the early 1970s (5). Resistant sorghum hybrids were developed that provided effective control of the disease (5); however, new pathotypes of the pathogen that are virulent to these previously resistant hybrids have necessitated a search for new sources of resistance (3,4).

Inoculating sorghum or corn seedlings in the greenhouse with conidia of *P. sorghi* is an effective method of identifying resistant genotypes (2,11). However, *P. sorghi* produces conidia only on the leaves of infected plants, and securing large amounts of mature ungerminated conidia presents difficulties. The environmental conditions required for good sporulation on the host plant (20–24 C and >85% relative humidity [RH]) permit rapid germination of mature conidia (1,8,11).

Two approaches have been used to devise inoculation techniques that cope with the problem posed by the evanescent viability of the conidia of *P. sorghi*. In one, infected leaves are placed on or above the sorghum seedlings in an environment that will induce sporulation (2,6,7). In the other, diseased sorghum plants or detached infected leaves are placed in an environment conducive to sporulation. Sporulation occurs and the conidia are collected, mixed with water, and sprayed on the test plants (1,11). Inoculation by spraying plants with a conidial suspension is preferable to other methods because it permits uniform application of a known quantity of inoculum (11). However, determining the length of the leaf-incubation period that will give the maximum yield of mature ungerminated conidia is difficult.

Safeeulla (10) reported a negative relationship between the duration of the incubation period required to induce sporulation and the duration of the light period to which the leaves had been subjected before incubation. He also noted that conidial production on leaves lasted about 4 hr regardless of the duration of the incubation period required to induce sporulation. Lal (9) reported that infected leaves incubated at 7:00 P.M. began producing conidia of *P. sorghi* after 9 hr of incubation. He noted that the conidia separated from the conidiophores when mature and germinated within 1 hr of maturation.

The characteristics of sporulation by *P. sorghi* noted above pose problems for producing and collecting conidial inoculum. Incubation periods of sufficient duration to secure maximum conidial production on donor leaves will result in germination of the conidia produced during the first 2 hr of a 4-hr period of conidial production. In addition, incubation intervals greater than 6 hr make it difficult to fit both conidial production and plant inoculation into a work day. Kimigafukuro and Leu (8), working with *P. sacchari*, the causal agent of sugarcane downy mildew, reported a similar problem in securing mature ungerminated conidia for use as inoculum. In studies on conidial preservation, they incubated diseased leaves of sugarcane above plates of water agar amended with $Mg(NO_3)_2$, NH_4Cl, or NH_4NO_3. At 12 hr after leaf incubation began, the conidia deposited on the surface of agar containing these salts remained ungerminated and germinated at rates of 60–80% when transferred to water agar. Traylor and Dunkle (12) tested the effects of antibiotics and metabolic inhibitors on conidia of *P. sorghi*. They reported that the conidial dormancy induced by these agents was irreversible.

Schmitt and Freytag (11) reported that conidia of *P. sorghi* required 24 hr to germinate when incubated at 6 C. Their results indicated that low-temperature inhibition of conidial germination could provide a solution to the problems of conidial production if a practical method

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of subjecting conidia to low temperatures at maturity could be devised.

This paper reports the results of a study on the inhibition of germination of the conidia of *P. sorghi* by low temperatures, effects of duration of storage at low temperature on viability of the conidia, and the development of a system for producing and preserving conidial inoculum of *P. sorghi*.

MATERIALS AND METHODS

A container for producing and storing conidia was constructed with a glass beaker 25 cm high and 16 cm in diameter (Fig. 1). The beaker was lined with a layer of absorbent cheesecloth. Fifty milliliters of distilled water was placed in the beaker to moisten the cheesecloth. A glass bowl 15 cm in diameter and 7.5 cm deep that contained 50 ml of 2% water agar was placed in the beaker. A disk of galvanized wire screen (6-mm mesh, 17 cm in diameter) was placed over the top of the beaker.

Infected leaves taken from sorghum plants in the early stages of systemic sorghum downy mildew were the source of conidia. Chlorotic leaves were cut from the diseased plants and placed abaxial side down on the wire screen. A pad of Kimpak seed germination paper (Seedburo Equipment Co., Chicago, IL) was saturated with distilled water, pressed to remove the free water, and placed over the leaves. Plastic wrap was placed over the pad to reduce evaporation.

The beaker was placed in a refrigerated water bath in a controlled-temperature chamber. The base of the beaker was 5 cm below the surface of the water. The air temperature and RH in the chamber were maintained at 21 ± 1 C and 95 ± 5%. This arrangement produced a situation in which the temperature and RH at the top of the beaker induced sporulation by *P. sorghi* on the infected sorghum leaves. The mature conidia separated from the conidiophores and fell to the water agar at the base of the beaker where agar temperatures of 5 C or lower inhibited germination of the conidia. At water bath temperatures of 1–4 C, temperatures of the agar were 1 C higher than water bath temperatures.

Trials were conducted to determine the effects of agar temperature and conidial production-storage interval (time interval between starting incubation of leaves and collecting conidia from agar surface) on conidial viability. Production-storage intervals of 16, 24, and 32 hr at agar temperatures of 3 and 5 C were compared. The production-storage intervals of 16 and 24 hr were compared in three trials at each of the two temperatures. In a second series of trials, production-storage intervals of 24 and 32 hr were compared.

At the end of the production-storage interval, the conidia were washed from the agar surface with 50 ml of a 0.02% solution of Tween 20 in distilled water. The conidial concentration and the frequency of germinated conidia at time of collection were determined by microscopic examination. The conidial concentration was adjusted to 4×10^4/ml. To determine conidial viability, drops of conidial suspension (30 µl per drop) were placed on the surface of 2% water agar in plastic petri dishes. Each drop was covered with a 25-mm^2 coverslip. The closed petri dishes were wrapped in moist paper and incubated in a controlled-temperature chamber at an air temperature and RH of 21 ± 1 C and 95–100%. Conidial suspensions of 4×10^4/ml were chosen to evaluate the germinability of the conidia, because preliminary tests indicated that germination of conidia was reduced at lower conidial concentrations (*unpublished*). After 20 hr of incubation, the conidia were observed microscopically for germination. A conidium with a germ tube longer than the width of the spore was considered germinated. The germination percentage of a given treatment was determined by observing 200 spores.

The conidia produced during a production-storage interval of 24 hr at agar temperature of 5 C were tested for ability to infect the susceptible corn inbred N28. The corn plants were grown in the greenhouse in peat pots, four plants per 6-cm pot, and inoculated at the 1.5- to two-leaf stage of growth. Conidial inoculum was produced as described. A conidial suspension of 2×10^4/ml was sprayed on the corn plants with an aerosol sprayer (Crown Spra-Tool, Fisher Scientific, Pittsburg, PA), at the rate of 0.5 ml per plant. Twenty-eight plants were inoculated and placed in a controlled-temperature chamber, where air temperature and RH were maintained at 20 ± 1 C and >95%. The plants were removed from the chamber after 24 hr and planted in 10-cm pots. The plants were grown in the greenhouse and observed for symptoms of downy mildew for 3 wk after inoculation. Plants that did not show symptoms within 3 wk were presumed to have escaped infection. The trial was repeated twice.

RESULTS AND DISCUSSION

The mean frequency of germinated conidia at time of collection was 1.7% for the 24-hr production-storage interval at 5 C. In the remaining five production-storage treatments, the mean frequencies for conidial germination at collection were less than 1%.

Increasing the duration of the production-storage interval from 16 to 24 hr did not reduce conidial viability (Table 1). The conidial germination percentages of 16- and 24-hr treatments ranged from 85 to 92% with no significant differences ($P = 0.05$) between treatments. Increasing the production-storage interval from 24 to 32 hr at 5 C reduced conidial viability. Conidial germination was significantly less ($P = 0.05$) in the 32-hr treatment (41%) than in the 24-hr treatment (88%).

The inoculation of N28 corn seedlings with conidia produced by a 24-hr production-storage interval induced a mean disease incidence of 99%. The viability and infectivity of conidia produced by a 24-hr production-storage interval are equivalent to those of freshly matured conidia. Schmitt and Freytag (11) tested conidia shortly after maturation and reported a germination percentage of 90% and 90–100% disease incidence in inoculated, susceptible corn seedlings. Although conidial germination percentages were not seriously reduced by production-storage intervals of 16 and 24 hr, the duration of incubation at 21 ± 1 C required to induce germination was

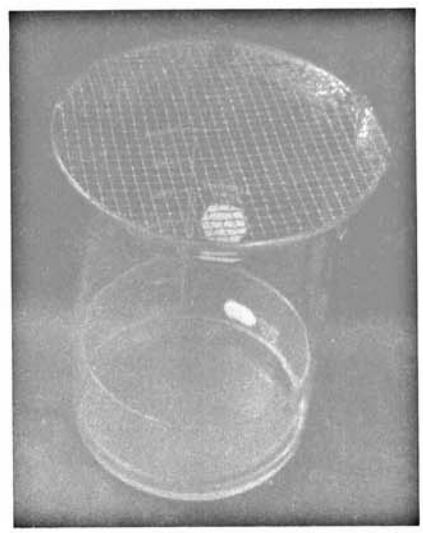

Fig. 1. Components of device for producing and storing conidia of *Peronosclerospora sorghi*: beaker (with cheesecloth liner removed), glass bowl containing water agar, and wire screen disk.

Table 1. Germination percentages of conidia of *Peronosclerospora sorghi* produced by three production-storage durations and two storage temperatures

Production-storage duration[a] (hr)	Conidial germination[b] (%) at each storage temperature (C)[c]	
	3	5
16 vs. 24 hr		
16	86	85
24	92	89
24 vs. 32 hr		
24	81	88
32	57	41*[d]

[a] Number of hours intervening between incubation of donor leaves and collection of conidia from water agar.

[b] Frequency of germination in conidia after 20 hr of incubation at 20 C; mean of three trials of each treatment.

[c] Temperature of water agar upon which conidia from donor leaves are deposited.

[d] * = Significant difference ($P = 0.05$) between germination percentages at 24 and 32 hr at 5 C determined by *t* test.

increased noticeably over that required to secure germination in conidia tested shortly after maturation. Conidia produced in the 16-, 24-, and 32-hr production-storage treatments required incubation periods of 4–6 hr to initiate germination. Schmitt and Freytag reported that freshly matured conidia incubated at 21 C began to germinate after less than 1 hr of incubation (11). The delayed germination of conidial inoculum does not present a problem. The inoculated plants are incubated for 24 hr after inoculation, which allows ample time for conidial germination.

The major advantages of this method of producing conidial inoculum are that it secures maximum production of conidia from the donor leaves and provides flexibility in timing the plant inoculations. I have used the system for several months to produce inoculum to test sorghum genotypes for resistance to *P. sorghi*. In this operation, the donor leaves are incubated at 4:00 P.M. the day before the planned inoculation, and the conidia are collected at any time between 8:00 A.M. and 4:00 P.M. on the day of the inoculation. The reactions of resistant and susceptible genotypes of sorghum and corn to conidial inoculum produced with the tiered temperature system were the same as those induced by inoculum obtained with previously reported methods (2,11; *unpublished*).

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