Consistent Infection of Corn Seedlings with Oospores of *Peronosclerospora sorghi*

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ABSTRACT

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Oospores of *Peronosclerospora sorghi* were produced in leaves of infected sorghum plants in the greenhouse. The leaves were harvested 5–6 wk after the plants were inoculated with conidia, dried at 35 C, and stored at 5 C. The dried leaves were shredded in a Waring blender and mixed with potting soil. Seeds of corn and sorghum were planted in the infested soil. The oospore inoculum consistently induced high incidences of sorghum downy mildew in susceptible corn genotypes, but was much less effective in tests with susceptible sorghum genotypes.

Sorghum downy mildew is an important disease of corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench.) in Asia, Africa, and the Americas (11). Host resistance is an effective means of controlling the disease. Resistance to *Peronosclerospora sorghi* (Weston and Uppal) C. G. Shaw, the causal agent of sorghum downy mildew, has been identified by exposing plants to the pathogen in field nurseries (6) or by conidial inoculation of young plants in the greenhouse (4).

The pathogen produces both conidia and oospores. Effective techniques for inoculating corn and sorghum with conidia have been devised (4,10,12,16), but no reliable method of inoculation with oospores has been reported. Uppal and Desai (17) described an oospore inoculation technique in which infected sorghum leaves were ground into powder and placed around seeds in the soil. Several researchers have used this technique or slight variations of it in studies of host plant reactions to *P. sorghi* (1,3,5,9,13–15,18). None of these investigators have reported consistent success with oospore inoculation and most have stated that test results were erratic or differed significantly from the results obtained in field nurseries (1,3,5,8,9,13,14).

A dependable procedure for inoculation with oospores would be useful. Conidial inoculation of plants in the greenhouse requires equipment to control temperature and RH. Lack of this equipment makes conidial inoculation impractical for some investigators. In addition, research on primary infection in the disease cycle of sorghum downy mildew requires the use of oospore inoculum.

In earlier studies of sorghum downy mildew, I conducted a series of trials with plants of susceptible corn genotypes by inoculating them with collections of oospores obtained from diseased sorghum plants found in the field. Oospores are produced abundantly in the leaves of systemically infected sorghum plants, but infrequently in corn (11). The incidence of infection was high in some trials and low in others. My observations indicated that the major cause for the variation among trials was a variation for infectivity among the collections of inoculum (5).

Possible causes of variation among the field collections of oospores were climatic conditions, amount of damage from microorganisms, and age of oospores at time of collection. Uniform conditions for the development, collection, and storage of oospore inoculum should reduce the variability among inoculum samples.

This paper describes a technique for the production and use of oospore inoculum, which induced consistent infection of susceptible corn genotypes.

MATERIALS AND METHODS

Oospore production. Germinated seeds of the commercial sorghum hybrid Tophand were inoculated with conidia of pathotype 1 of *P. sorghi* (7) by placing leaves of diseased plants above the seeds shortly after germination (10). The inoculated seedlings were planted four per pot, in 6-cm-diameter peat pots, and grown in the greenhouse for 10 days. Symptomless plants were removed from the pots, and the pots of diseased plants were planted in 10-cm-diameter clay pots. Within 3 wk after inoculation, the younger leaves produced by the diseased plants displayed the streaks of chlorotic, interveinal tissue indicative of oospore formation (8).

At 5–6 wk after inoculation, the diseased plants were cut off at soil level and dried in a mechanical convection oven for 5 days at 35 ± 2 C. The dried tissue was cut into sections approximately 1 cm long and stored at 5 ± 2 C. Eight populations of diseased sorghum plants were grown in succession to produce eight separate lots of oospore inoculum.

Corn inoculation. Three lots of oospore inoculum were selected at random and tested for ability to infect the susceptible inbred corn line N28 (6). Peat pots (6-cm-diameter) were filled to 1 cm below the top with a moist, pasteurized mixture of one part (v/v) Baccto potting media (Michigan Peat Co., Houston, TX 77006), three parts peat moss and three parts soil. Six corn seeds, which had been incubated on wet paper at 28 ± 1 C for 16 hr, were placed, scutellum side up, on the surface of the potting medium in each pot.

Samples of dried plant material from each of the three lots of inoculum were shredded in a Waring blender for four 1-sec intervals. The shredded material from each inoculum lot was mixed with air-dry potting medium at the rate of 3 g of plant material per 100 cm^3 of potting medium, and 20 ml of distilled water per 100 cm^3. Thirty corn seeds (five pots) were inoculated for each lot of inoculum by covering the seeds with 10 cm^3 of moist, infested potting medium. The pots were then filled to the top with moist, pasteurized, potting medium. Seeds of N28 were planted in pots of moist, pasteurized potting medium as a control treatment.

The peat pots were placed in plastic trays in the greenhouse. Distilled water was added to the trays to a depth of 1 cm; care was taken to avoid pouring water on the surface of the potting medium. The depth of distilled water in each tray was restored to 1 cm at

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24-hr intervals.

The peat pots of corn seedlings were planted in 10-cm-diameter clay pots 7 days after inoculation. The plants were grown in the greenhouse and inspected daily for symptoms of sorghum downy mildew. The diseased plants were removed from the pots when symptoms appeared. Plants that did not show symptoms within 30 days after planting were assumed to have escaped infection. The trial was repeated twice.

Three resistant corn inbred lines (33-16, R177, and W117) and the susceptible inbred N28 were inoculated to test the ability of the inoculation procedure to identify resistance to *P. sorghi*. The inbreds, 33-16, R177, and W117 had exhibited resistance to sorghum downy mildew in repeated field trials in Texas. The corn inbreds were inoculated with an inoculum lot chosen at random from the three lots tested with N28. The inoculation procedure was the same as described above. The trial was repeated once.

The five remaining collections of oospore inoculum were tested for ability to infect the susceptible corn inbred B68 (6). The inoculation procedure was the same as described above. These tests were repeated once.

Sorghum inoculation. An inoculum lot that had induced a disease incidence of 100% in B68 was used to inoculate the susceptible sorghum inbred line Tx412. The inoculation procedure was the same as for corn except that seeds were incubated for 6 hr, a layer of 10 cm^3 of infested potting medium was placed below the seeds as well as above, and orientation of the scutellum was disregarded. Seeds of B68 were inoculated as a control treatment. The trial was repeated twice.

The mean percentage of infection induced in N28 by the three lots of inoculum in three trials was determined and the coefficient of variability for the nine test observations was calculated. The same procedure was applied to the results of the test of lots 4, 5, 6, 7, and 8 with B68 and of lot 4 with Tx412.

The results of the two trials of the reactions of three resistant corn lines and N28 were compared. No significant difference was found between the trials, and the results of the two trials were combined. The infection percentages of the corn lines were calculated and compared with the percentages of infection observed on these inbreds in field trials.

TABLE 1. Incidence of sorghum downy mildew induced in corn inbreds B68 and N28, and sorghum inbred Tx412 by oospore inoculum of *Peronosclerospora sorghi*

	Infection[b] (%)			
Inoculum lots[a]	N28	B68	Tx412	CV[c] (%)
None	0[d]	···	···	···
Lots 1, 2, and 3	94[d]	···	···	8
Lots 4, 5, 6, 7, and 8	···	95[e]	···	7
Lot 4	···	100[d]	14[d]	73

[a] Collections of inoculum produced at different dates in the greenhouse.
[b] Mean percentage of plants with sorghum downy mildew 30 days after planting.
[c] Coefficient of variability of test results. The CV of Lot 4 inoculum refers to Tx-412 results only.
[d] Mean of three trials, with 30 plants inoculated per inoculum lot in each trial.
[e] Mean of two trials, with 30 plants inoculated per inoculum lot in each trial.

TABLE 2. Reactions of corn inbred lines to *Peronosclerospora sorghi* in field nurseries and oospore inoculations in the greenhouse

	Infection[a] (%)	
Inbred	Field[b]	Oospore inoculation[c]
N28	100	89
33-16	6	2
R177	5	2
W117	2	13

[a] Percentage of plants with sorghum downy mildew.
[b] Maximum percentage of diseased plants observed in three or more field trials.
[c] Mean of two trials of 30 inoculated plants per inbred.

RESULTS AND DISCUSSION

The eight collections of inoculum induced consistently high levels of disease in the susceptible corn inbred lines (Table 1). The incidences of sorghum downy mildew observed in these tests were similar to those observed on these inbreds under field conditions favorable to the disease. The uniform infectivity of the inoculum collections was demonstrated by the small coefficients of variability associated with the results of the corn tests (Table 1).

In contrast, the inoculation tests of the sorghum line Tx412 produced a high level of variability among test results (Table 1). The test results were not representative of the susceptibility of Tx412, which often had 80–100% infection in field trials. Additional inoculation trials conducted with other sorghum inbreds and hybrids, including hybrid Tophand, were as ineffective as those with Tx412. The cause of these failures to induce SDM in susceptible sorghum is unknown, but the level of infection induced in the B68 corn used as the control treatment in the test of Tx412 (Table 1) indicated that quality of the inoculum was not a factor.

The inoculation trials of the resistant corn inbreds, 33-16, R177, and W117, and the susceptible N28 gave results similar to those attained in field trials of these inbreds (Table 2).

The eight collections of inoculum differed in the length of the storage period preceding tests for infectivity. The storage intervals ranged from 3 wk to 13 mo; no relationship was found between length of storage interval and infectivity of inoculum. The amount of dried, infected plant tissue used to infest the potting medium with oospores was determined by the results of previous infectivity tests of each lot of inoculum at rates of 1, 2, 3, and 4 g of plant tissue per 100 cm^3 of potting medium. The infestation ratio chose for this study, 3 g of plant material to 100 cm^3 of potting medium, was the lowest rate at which all lots of inoculum induced 90% or more infection in the corn inbred B68.

No attempt was made to determine the number of oospores per gram of infected plant tissue. The establishment of infestation levels on the basis of weight of plant tissue rather than numbers of oospores was preferable because of its quickness and simplicity. Research workers who use this inoculation technique should determine the effective rates of infestation for their collections of inoculum by testing a range of rates for infectivity to a susceptible corn genotype. The number of tests can be minimized by dealing with a few large lots of inoculum rather than several small lots.

The inoculation procedure used in this study represented an attempt to combine factors favorable for infection by *P. sorghi*. High soil moisture was reported to be detrimental to infection by oospores (2). The mixture used for the potting medium and the restricted application of water during the first 7 days after inoculation were designed to prevent high soil moisture. The seeds were incubated on moist paper before planting to insure rapid and uniform germination despite the low soil moisture of the potting medium.

The potting medium was air-dried before infestation to facilitate the mixing of medium and inoculum. The purpose of shredding the dried leaves was to produce small particles of leaf debris that mixed easily with the potting medium, not to free the oospores from leaf tissue. The length of the shredding period was kept short to reduce mechanical damage to the oospores.

The techniques for oospore production and inoculation described here induced consistent infection in susceptible corn genotypes and differentiated corn inbreds resistant to sorghum downy mildew. Production of dependable oospore inoculum provides the means to pursue some previously impractical lines of research on sorghum downy mildew. Among these are studies on differences between host reaction to conidia and to oospores, and the effects of environment on oospore infectivity.

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