Symptoms of Sorghum Downy Mildew on Maize Following Inoculations with Conidia and Oospores

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ABSTRACT

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The response of four selected maize inbred lines inoculated in the greenhouse using conidia and oospores of *Peronosclerospora sorghi* was evaluated. Reaction of specific cultivars to infection with different spore forms of *P. sorghi* is important in screening for host resistance. The inbred Tx601 was resistant to both types of inoculum, whereas Tx441 was more susceptible to conidia than oospores. Irrespective of reaction class, systemically infected plants had different colonization symptoms.

Additional key word: corn

In the last 20 yr, sorghum downy mildew (SDM), caused by the fungus *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw, has become the most widely distributed tropical downy mildew, causing heavy damage to *Sorghum* spp. and *Zea mays* L. in many countries (9). Since the first report of SDM in the United States in 1961 (12), the disease has spread rapidly. In 1969, losses by the pathogen in sorghum, broomcorn, and maize were estimated to be $2.5 million in Texas (7). Since first reported, the disease has spread to adjacent states and reached as far north as Indiana and Nebraska (11,13).

P. sorghi colonizes its host either locally or systemically. Systemic infection originates from soilborne oospores or from conidial infection shortly after seedling emergence. Occasionally, oospores are formed in maize leaf and tassel tissue between veins (7). These survive as resting spores in the soil and crop residue for several years. Conidia produced on the abaxial side of the leaf following systemic infection are short-lived (8).

Techniques for inoculating sorghum and maize using conidia (10) and oospores (7) as inoculum have been described. These techniques have been used to study the infection process and screen for host resistance. The most effective means of controlling the disease has involved deployment of resistant cultivars (9).

This paper describes the reactions of four selected maize seedlings to the SDM pathogen and their response to infection by conidia and oospores.

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MATERIALS AND METHODS

Four maize inbred lines (Tx601, Tx441, Tx127C, and Tx508) ranging from resistant to susceptible, respectively, were used in the field.

Conidial inoculation. Conidial inoculation procedures were as described by Jones and Frederiksen (10) for increase of inoculum and that designed by Craig (5) for inoculation of maize seedlings. Inoculum was increased on an SDM-susceptible cultivar of *S. bicolor* (L.) Moench. The sorghum seeds were germinated in petri dishes at room temperature. Seeds were hydrated and incubated for approximately 24 hr. Germinated seeds were placed embryo side up on moist paper in petri dishes; infected sorghum leaves freshly harvested late in the afternoon were placed on top of one layer of cheesecloth with the lower leaf surface downward above the germinating seeds, covered with moist paper in closed petri dishes, and placed in a moist atmosphere at 21 C overnight. Seeds were then planted in 5.7-cm peat pots, six seeds per pot, and kept in the greenhouse in polystyrene trays 7–15 days. These infected sorghum plants were used as an inoculum source.

Conidial inoculation on maize seedlings was performed as follows: seeds were germinated by incubation in a petri dish on moist germination paper at 29 C for 2–3 days. Five seeds per pot were planted into 5.7-cm peat pots of steam-sterilized, commercial potting soil. Plants were grown in polystyrene trays up to approximately 7 days (full exposure of the first true leaf) and then inoculated. The inoculation chamber was made of two polystyrene trays 8.7 cm deep × 32 cm long. The peat pots containing seedlings were placed in the lower tray, which was half filled with water to maintain high relative humidity in the chamber. During the inoculation period, a hole 13.9 × 16.5 cm was cut in the base of the second tray, which was placed over the tray with the seedlings.

Twenty to 30 sorghum leaves from the source infected with downy mildew were

cut into 2-cm sections and placed abaxial side down between cheesecloth and a moist pad over the wire screen covering the hole in the upper tray. The inoculation chamber was kept at 20–21 C in a 100% saturated atmosphere. Conidia were produced 6–8 hr after onset of the incubation period and continued for 2–4 hr. Compressed air delivered by a pump at about 3,500 kg/m^2 (air-line pressure) scattered the conidia produced on the leaves in the upper tray over the seedlings in the lower tray. Inoculated plants were planted in 12-cm plastic pots of commercial potting soil and kept 28 days for observation.

Oospore inoculation. Oospores were collected as follows: shredded sorghum-sudan hybrid leaves were dried and midveins removed, ground in a Wiley mill, and sieved through a 200-mesh sieve (75-μm openings). The resulting finely ground powder containing oospores was stored at 4 C until used as inoculum.

Ships clay soil obtained from the Texas A&M plantation farm at College Station was used in the experiments. Unsterilized soil collected from a field with a history of SDM near Beeville, TX, was also used to observe plant infection from different sources of oospore inoculum. The soil was screened through 1-cm mesh wire and steam-sterilized for 60 min at 120 C at 1.05 kg/cm^2.

Seeds were moistened, coated with oospores, and planted in stainless steel trays containing approximately 2,200 cc of sterilized soil. The trays were placed into an environmental chamber maintained at 29 C for 3 days and then transferred to the greenhouse. Observations for symptoms of downy mildew were taken, and percentage of infected plants was determined during a 28-day period.

RESULTS

Conidial inoculation. Local lesion symptoms developed on all lines 6–8 days after inoculation with conidia. Tx441 and Tx508 were the first to show symptoms, and Tx601 was the last. Local lesions appeared on leaves as somewhat large chlorotic areas expanding parallel with the veins (Fig. 1A), elongated chlorotic spots extending from the bottom to the tip, or more narrow elongated chlorotic spots restricted between veins. Lesions were either localized on lower leaves or the lesions extended downward on the inoculated leaf and caused systemic symptoms (Fig. 1B). Generally, systemic symptoms appeared on third and fourth leaves 7–8 days after inoculation and were present on all young leaves emerging from the whorl. Sometimes systemically infected leaves appeared without local lesions.

Systemically infected leaves exhibited different symptom patterns on different maize inbred lines: chlorotic leaves on Tx508 and Tx601 (Fig. 1C), a mosaiclike pattern of spotting and mottling on Tx441, and necrotic-chlorotic lesions on Tx127C (Fig. 1D).

The incidence of infected plants after conidial inoculation is summarized in Table 1. Significant differences in susceptibility were obtained among inbreds. Based on these evaluations,

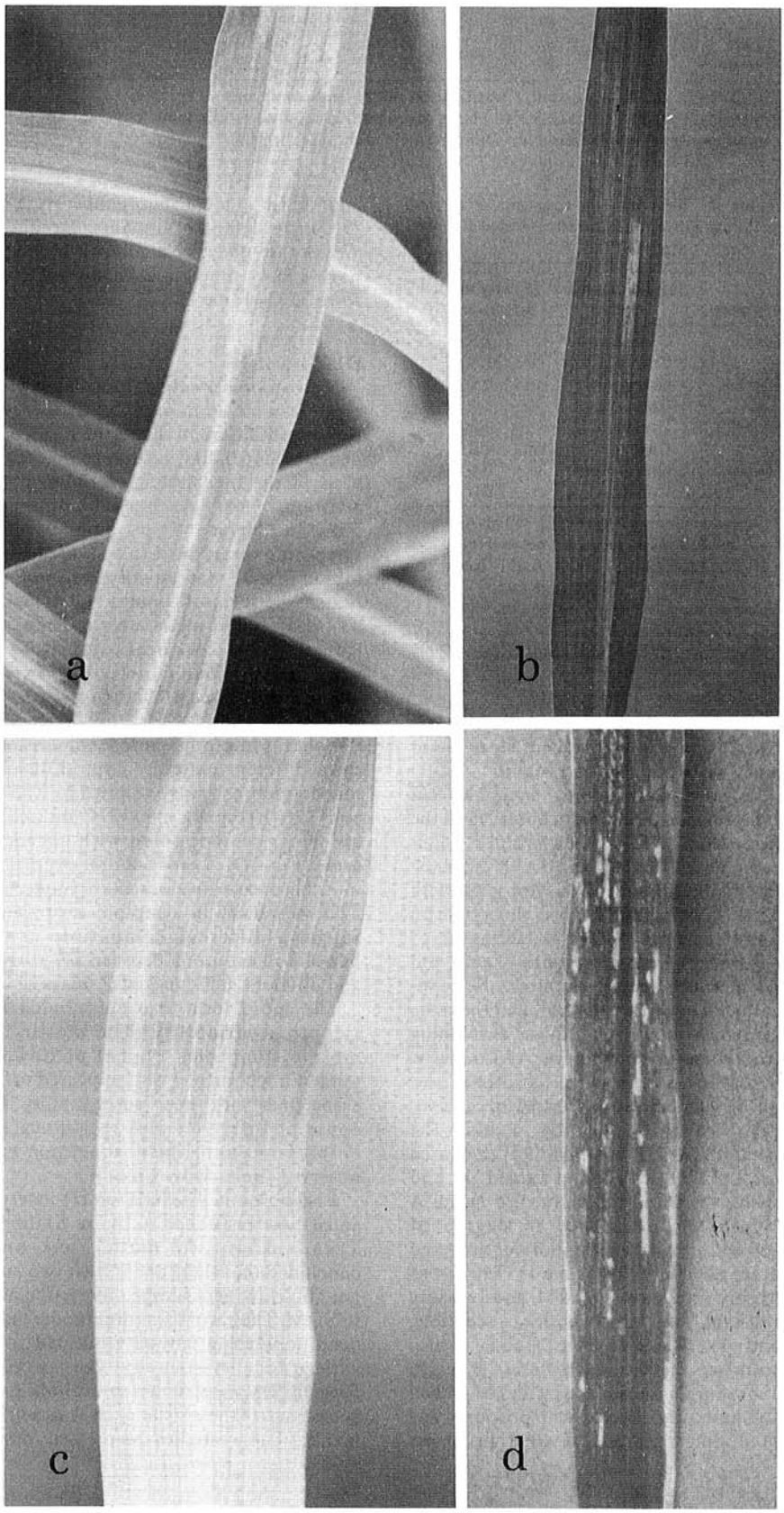

Fig. 1. Maize leaves infected with *Peronosclerospora sorghi*: **(A)** Local lesions on Tx508 appearing as a large chlorotic area expanding parallel with the veins. **(B)** Local lesions on Tx508 overlying systemic symptom. **(C)** Systemic infection with general chlorosis. **(D)** Mottling on leaves of systemically infected Tx127C.

Table 1. Comparison of the effect of different spore forms of *Peronosclerospora sorghi* on disease incidence in seedlings of four maize inbred lines

Cultivar	Plant reaction		Downy mildew infection (%)[z]	
	Conidia	Oospores[y]	Conidia	Oospores
Tx508	S	S	66.6 b	55.2 b
Tx127C	R	S	12.5 a	32.2 b
Tx441	S	R	42.3 b	11.2 a
Tx601	R	R	0.5 a	0.0 a

[y] S = susceptible, R = resistant. Classification under field conditions.
[z] Means of four replicates of 30–40 plants each. Values followed by the same letters do not differ significantly ($P = 0.05$) based on Duncan's new multiple range test.

Table 2. Reaction of four maize inbred seedlings grown on naturally infested soil with *P. sorghi* oospores

Cultivar	Soil temperature control	Downy mildew infection (%)[x]
Tx508	Greenhouse[y]	7.5 b
	Constant[z]	21.8 a
Tx127C	Greenhouse	0.9 b
	Constant	9.8 b
Tx441	Greenhouse	0.0 b
	Constant	2.2 b
Tx601	Greenhouse	0.0 b
	Constant	0.0 b

[x] Means of four replicates of 30–40 plants each. Values followed by the same letters do not differ significantly ($P = 0.05$) based on Duncan's new multiple range test.
[y] Temperatures ranged from 15 C at night to 35 C during the day.
[z] Maintained at constant temperature of 29 C.

Tx508 and Tx441 were susceptible whereas Tx127C was moderately resistant and Tx601 was highly resistant.

Oospore inoculation. Systemic leaf symptoms in plants were observed in the first true leaf 6–8 days after plant emergence. Tx127C and Tx508 were the first to develop infection symptoms, with Tx127C being the earliest. Infection on Tx441 became evident as late as 12–15 days after plant emergence. Tx601 did not develop systemic symptoms. Systemically mildewed leaves exhibited the same symptom patterns as those developing from conidial inoculations. Additionally, diseased plants were stunted and possessed narrower and more erect leaves than leaves on healthy plants. The stunting pattern was greatly accentuated on Tx127C, with leaves curled up and shriveled. Often the plant died early. A significant difference in degree of susceptibility and resistance was observed among the inbreds (Table 1). Tx601 was highly resistant, Tx441 moderately resistant, Tx127C moderately susceptible, and Tx508 susceptible. Table 1 also compares the effects of different *P. sorghi* spore forms on disease incidence. When soil naturally infected with oospores was used, infection was favored in plants maintained at a constant temperature of 29 C in a soil tank over plants placed in a greenhouse under semicontrolled conditions where temperatures fluctuated from 15 to 35 C (Table 2).

DISCUSSION

Maize inbred seedlings inoculated with conidia and oospores of *P. sorghi* responded differentially to the incidence of SDM depending on the spore forms used in the inoculation procedures. In addition, inbred lines responded differentially in degree of susceptibility and symptom expression (Table 1). The maize inbred Tx601 was equally resistant to both spore forms, oospores and conidia. Tx441 appeared moderately resistant to infection by oospores, but it was susceptible to conidial infection. Tx508 was the most susceptible to both types of inocula.

Foliar symptoms were expressed in three different patterns. Typical downy mildew chlorosis was exhibited by Tx508 and Tx601 (Fig. 1C). Tx127C showed a chlorosis accompanied with necrotic areas (Fig. 1D). Tx441 exhibited spotted, mottling areas with mosaiclike symptoms. This variability in symptom expression suggests different compatible host-parasite interactions between (*P. sorghi* and different genotypes of *Z. mays*.

The spore form also contributed to symptom variability. The mottle or mosaic symptoms typical of downy mildew infection of Tx441 appeared to be associated with host susceptibility to conidial infection and resistance to oospore infection. This phenomenon was observed similarly by Craig (6).

Bockholt and Frederiksen (1) investigated host resistance to maize SDM in Texas using two diallel sets and concluded that there are at least two and possibly three genes controlling resistance to SDM. Their work was done under field conditions using naturally infested soil. Inheritance studies by Borges and Riccelli (2) suggested that several additive genes control the resistance to *P. sorghi* in maize. These studies were also done under field conditions. However, Chang et al (4) evaluated 29 composite maize varieties for SDM resistance in Thailand and Taiwan and suggested that the pathogenicity of *P. sacchari* and *P. sorghi* might be closely related. Chang and Cheng (3) proposed the existence of only one pair of dominant genes governing resistance to *P. sacchari* in maize.

These observations suggest that the mechanism of resistance to infection or colonization by germinating conidia differs from that of resistance to oospores, a position consistent with research observations by Craig (6). Because genetic control of each resistance mechanism may also differ, genes controlling resistance to conidia may not operate against oospores. Further genetic studies will be required to determine the inheritance of the reaction of maize to conidial or oospore infection by *P. sorghi*.

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