Comparative Reactions of Corn Inbreds to Oospore and Conidial Inoculum of *Peronosclerospora sorghi*

Jeweus Craig

Research plant pathologist, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Plant Sciences Department, Texas A&M University, College Station 77843.
Cooperative investigations of the Science and Education Administration, USDA, and Texas Agricultural Experiment Station, College Station. Approved for publication as Technical Article 15204 of the Texas Agricultural Experiment Station.
Accepted for publication 12 October 1979.

ABSTRACT

CRAIG, J. 1980. Comparative reactions of corn inbreds to oospore and conidial inoculum of *Peronosclerospora sorghi*. Phytopathology 70:313-315.

Corn inbreds that differed in reactions to infection by oospores of *Peronosclerospora sorghi* in field trials for susceptibility to sorghum downy mildew were tested for reactions to conidia of *P. sorghi*. The cultivars were inoculated with conidia at germination and at the first-, second-, and third-leaf stages of growth. All inbreds were susceptible at germination. Inbreds that were resistant to oospores developed resistance to conidia at or before the second leaf stage. Inbreds susceptible to oospores in the field were susceptible to conidia at the second-leaf stage. The percentages of downy mildew in corn inbreds inoculated with conidia at the second-leaf stage were closely correlated with the percentages of downy mildew induced by natural infection of these inbreds in the field.

Peronosclerospora sorghi (Weston & Uppal) C. G. Shaw (12), the causal agent of sorghum downy mildew (SDM), produces two forms of inoculm, oospores and conidia. Research workers have reported some lines of corn (*Zea mays* L.) to be more susceptible to conidia than to oospores of *P. sorghi* (3,4,6,8,11). Frederiksen and Renfro (3) noted two types of resistance to SDM in corn. One type was effective against oospores and conidia; the other was effective against oospores, but ineffective against conidia. Schmitt and coworkers (10) compared the reactions of corn lines to conidial inoculum with their reaction to field inoculum, presumably oospores. They concluded that resistance to the two types of inoculum was conditioned by similar genetic systems.

Any difference between the reactions of a corn line to oospores and conidia of *P. sorghi* increases the difficulty of selection for SDM resistance. Conidial inoculation (1) of corn plants in the greenhouse is a quick and relatively inexpensive method of screening for resistance to SDM. However, if conidial inoculation does not differentiate all types of resistance to SDM from susceptibility, its use will result in a deleterious reduction of the germplasm base for resistance to SDM. In many areas, any effective resistance to oospores would provide satisfactory control of SDM in field plantings.

Unfortunately, the alternatives to conidial inoculation for identification of SDM resistance are unsatisfactory. No reliable method has been devised for greenhouse tests of resistance with oospore inoculum. Disease nurseries planted in areas where SDM is endemic have been widely used (2). However, use of these nurseries is limited to the growing season, and the erratic occurrence of the disease requires repeated tests to ensure reliable identification of resistant lines.

In earlier studies, I noted that corn plants became less susceptible to conidial inoculum with increased age. This suggested that factors for resistance that were ineffective at an early stage of plant development could be identified by conidial inoculation at later stages of plant growth.

This paper presents the results of a study of the relationships between the reactions of corn inbreds to oospore inoculum and their reactions to conidial inoculation at different stages of growth.

MATERIALS AND METHODS

The reactions of several corn inbreds to *P. sorghi* were determined by repeated field trials in Texas (Table 1). Inoculum at the test sites consisted of oospores produced in the preceding crop of infected sorghum. The absence of conidial inoculum during the early stages of plant growth and the relatively concurrent appearance of disease symptoms indicated that the test results represented reactions to oospore inoculum.

Four inbred corn lines, B68, Oh43, Va26, and Tx601, were selected to test the effect of growth stage on reaction to conidial inoculum. The lines included a range of reactions to oospores: highly resistant (Tx601), moderately resistant (Oh43, Va26), and susceptible (B68) (2,10). The corn inbreds were compared for susceptibility to conidial inoculum at four growth stages: 16 hr or less after seed germination (GS0), 16 hr or less after the appearance of the auricle of the first leaf (GS1), 16 hr or less after the appearance of the auricle of the second leaf (GS2), and 16 hr or less after the appearance of the auricle of the third leaf (GS3). Each growth-stage trial was conducted in a completely randomized experimental design with four treatments (inbreds) and 12 plants per treatment. Each growth-stage trial was repeated once.

For tests of reactions to conidial inoculum at GS0, germinated corn seeds were placed, embryo side up, on moist tissue paper in a tray and covered with cheesecloth. Infected leaves taken from young sorghum plants in the early stages of systemic SDM were the source of conidial inoculum (1). Disks 1 cm in diameter were cut from the infected leaves with a cork borer. One leaf disk was placed, abaxial side down, on the cheesecloth directly above the coleoptile of each seedling. The leaf disks were fixed in place by thrusting a small pin through the leaf disk and into the seed endosperm; care was taken to avoid injuring the seedling.

The seedlings were held for 22 hr at 20 ± 3 C and relative humidity (RH) above 95%. After 22 hr, the lower surface of each

TABLE 1. Reactions of corn inbreds to *Peronosclerospora sorghi* in field trials

Inbred	Number of trials	Infection range (%)[a]
A632	4	17–61
B68	4	55–100
Mo17	4	0–22
N7A	3	0–95
Oh43	5	0–19
Tx601	6	0–0
Va26	4	2–24
W64A	4	0–41
33-16	4	0–6

[a]Minimum and maximum percentages of sorghum downy mildew-infected plants observed in trials.

leaf disk was examined macroscopically for sporulation by the pathogen. Seedlings under the leaf disks that showed signs of sporulation were planted (one plant per pot) in 10-cm-diamter clay pots of sterilized soil. The plants were grown in the greenhouse for 30 days and observed for the leaf chlorosis caused by systemic SDM (1).

For tests of reaction to conidial inoculum at GS1, GS2, and GS3, corn seeds were germinated in an incubator and planted (two seedlings per pot) in 6-cm peat pots of sterilized soil. The plants were grown in the greenhouse to the stage desired for inoculation and thinned to one per pot. A small piece of cheesecloth was placed over the base of the leaf blade of the youngest leaf with an auricle, eg, the third leaf in the GS3 trials. An infected leaf disk, obtained as described for GS0 inoculations, was placed abaxial side down on the cheesecloth and fastened in place with a pin through the leaf disk and the leaf blade. The plants were kept at 20 ± 3 C and RH >95% for 22 hr. This method of inoculation was devised to reduce variability in the location of the inoculated area on the plant and to ensure recognition of plants that had not been inoculated. After 22 hr, the leaf disks were examined for sporulation and the plants with leaf disks that showed signs of sporulation were planted in 10-cm-diameter clay pots of sterilized soil. The inoculated plants were grown in the greenhouse for 30 days and scored for systemic SDM.

The results of the first and second series of tests were similar, and the error variances of the two tests at each growth stage were homogeneous. The test results were combined for analysis and significant differences among the treatments were determined by single-degree-of-freedom analysis (13).

The placement of leaf disks on individual plants for conidial inoculation was laborious and unsuited to the simultaneous evaluation of large numbers of plants. Nine corn inbreds (Table 1) were used to determine whether equivalent results could be obtained with a mass inoculation technique. In this method of inoculation, the conidia were distributed at random over the plants by air currents (1). The corn plants were inoculated at the second leaf stage and scored for systemic SDM at 30 days after inoculation.

TABLE 2. Reactions of corn inbreds to inoculation with conidia of *Peronosclerospora sorghi* at different stages of growth

Inbred	Infection (%)[x] at indicated growth stage[y]			
	0	1	2	3
B68	100 a	100 a	96 a	95 a
Oh43	100 a	65 b	22 b	0 b
Va26	90 a	65 b	22 b	0 b
Tx601	45 b	20 c	0 b	0 b
r^z =	+0.60	+0.89	+0.99**	+0.97*

[x]Means of two replications of 12 inoculated plants per inbred. Within columns, numbers followed by same letter are not significantly different ($P = 0.05$) with single degree of freedom analysis.

[y]Growth stage at inoculation, 0 = germination, 1 = first leaf stage, 2 = second leaf stage, 3 = third leaf stage.

[z]Coefficient of correlation between infection percentages from inoculation and maximum infection percentages in field trials (Table 1); ** and * indicate statistical significance, $P = 0.01$ and $P = 0.05$, respectively.

TABLE 3. Reactions of nine corn inbreds to mass inoculation with conidia of *Peronosclerospora sorghi* at the second leaf stage of growth

Inbred	Number inoculated	Infection (%)[a]
A632	36	75
B68	57	97
Mo17	21	19
N7A	39	100
Oh43	23	4
Tx601	44	5
Va26	18	17
W64A	30	67
33-16	30	7
r^b = +0.957**		

[a]Percentage of inoculated plants with systemic sorghum downy mildew 30 days after inoculation.

[b]Coefficient of correlation between infection percentages from inoculation and maximum infection percentages in field trials (Table 1); ** indicates statistical significance, $P = 0.01$.

RESULTS

Lines Oh43, Va26, and Tx601 were more susceptible to infection by conidia at GS0 and GS1 (Table 2) than they were to infection by oospores in field trials (Table 1). These three field-resistant lines became less susceptible to infection by conidia at later growth stages. However, SDM resistance appeared earlier and reached a maximum earlier in Tx601 than in Oh43 and Va26. Line B68, the field-susceptible entry, was very susceptible to conidial inoculum at all the growth stages.

The percentages of SDM-infected plants in these lines after conidial inoculation at GS0 and GS1 were not significantly correlated with the incidence of SDM observed in field nurseries. However, significant positive correlations were found between the response of cultivars to field inoculum and their response to conidial inoculum at GS2 and GS3 (Table 2).

The mass inoculation of corn at GS2 gave results similar to those obtained with individual plant inoculations (Table 3). The SDM levels in the inoculated inbreds had a strong positive relationship with the maximum levels of infection observed in the field.

DISCUSSION

The systemic phase of SDM is caused by the colonization of meristematic foliage by *P. sorghi*. Previous reports (5,14) indicated that factors that inhibited the pathogen's growth were not present in meristematic tissues but developed as these tissues matured. This conclusion was supported by the high percentage of systemic SDM in inbreds inoculated at germination (Table 2). The decreased susceptibility exhibited by Oh43, Va26, and Tx601 at later stages of growth suggests the presence of inhibitory factors in maturing leaf tissues. This barrier of inhibition, interposed between the inoculated area and susceptible meristematic tissue, reduced systemic infection. The susceptibility of B68 at all growth stages indicated that factors inhibitory to *P. sorghi* are not produced by this line.

It was assumed that the highest percentage of SDM observed on these lines in field trials (Table 1) represented their maximum susceptibility to oospore inoculum. If this is true, oospores and conidia differed in ability to induce SDM in Oh43, Va26, and Tx601 at the early stages of plant growth. This difference may be related to differences between the spore forms in time and site of host penetration. Conidia invade the aerial plant parts. Oospore invasion is restricted to the roots (9). It is unlikely that oospores invade the roots either at or soon after germination. Pratt (7) reported that oospores germinated only in the presence of growing roots. The time required for the growth of roots, stimulation of oospore germination, and penetration of roots would permit considerable growth by the seedling before invasion. This growth would increase the distance between the meristematic foliage and the point of pathogen penetration. In addition, the maturation of root and mesocotyl tissue during this period may retard the progress of the pathogen. The correlations between reactions to field inoculum and to conidial inoculum (Table 3) suggest that the interactions between the corn lines and oospores were similar to the interactions between these inbreds and conidia at the second leaf stage of growth.

The results of this study indicate that the reactions of corn lines at the second-leaf stage to conidial inoculum can be used to predict their relative susceptibility to oospore inoculum under field conditions. Consequently, greenhouse tests of corn lines with a mass inoculation technique (1) apparently can be substituted for the more expensive and unreliable field trials.

LITERATURE CITED

1. CRAIG, J. 1976. An inoculation technique for identifying resistance to sorghum downy mildew. Plant Dis. Rep. 60:350-352.
2. CRAIG, J., A. J. BOCKHOLT, R. A. FREDERIKSEN, and M. S.

ZUBER. 1977. Reactions of important corn inbred lines to *Sclerospora sorghi*. Plant Dis. Rep. 61:563-564.
3. FREDERIKSEN, R. A., and B. L. RENFRO. 1977. Global status of maize downy mildew. Annu. Rev. Phytopathol. 15:249-275.
4. FREDERIKSEN, R. A., and A. J. ULLSTRUP. 1975. Sorghum downy mildew in the United States. Trop. Agric. Res. 8:39-46.
5. KENNETH, R. G. 1976. The downy mildews of corn and other Gramineae in Africa and Israel, and the present state of knowledge and research. Kasetsart J. 10:148-159.
6. KENNETH, R. G., and G. SHAHOR. 1973. Systemic infection of sorghum and corn by conidia of *Sclerospora sorghi*. Phytoparasitica 1:12-21.
7. PRATT, R. G. 1978. Germination of oospores of *Sclerospora sorghi* in the presence of growing roots of host and nonhost plants. Phytopathology 68:1606-1613.
8. SAFEEULLA, K. M. 1976. Sorghum downy mildew of maize in Karnataka, India. Kasetsart J. 10:128-134.
9. SAFEEULLA, K. M. 1976. Biology and Control of the Downy Mildews of Pearl Millet, Sorghum and Finger Millet. Wesley Press, Mysore, India. 304 pp.
10. SCHMITT, C. G., G. E. SCOTT, and R. E. FREYTAG. 1977. Response of maize diallel cross to *Sclerospora sorghi*, cause of sorghum downy mildew. Plant Dis. Rep. 61:607-608.
11. SHABANI, S. 1978. Reaction of *Zea mays* L. to inoculation with *Sclerospora sorghi* Weston & Uppal. M.S. Thesis, Texas A&M University, College Station. 59 pp.
12. SHAW, C. G. 1978. *Peronosclerospora* species and other downy mildews of the Gramineae. Mycologia 70:594-604.
13. SNEDECOR, G. W. 1940. Statistical Methods. 3rd ed. Iowa State College Press, Ames. 485 pp.
14. YEH, Y. 1977 Histopathology of *Sorghum bicolor* (L.) Moench. resistant and susceptible to the infection of *Sclerospora sorghi* (Kulk.) Weston & Uppal. M.S. Thesis, Texas A&M University, College Station. 54 pp.