Survival of *Colletotrichum graminicola* Sclerotia in Sorghum Stalk Residues

C. R. CASELA, Former Graduate Student, and R. A. FREDERIKSEN, Professor, Department of Plant Pathology & Microbiology, Texas A&M University, College Station 77843-2132

ABSTRACT

Casela, C. R., and Frederiksen, R. A. 1993. Survival of *Colletotrichum graminicola* sclerotia in sorghum stalk residues. Plant Dis. 77:825-827.

Sclerotia of *Colletotrichum graminicola*, the causal agent of sorghum anthracnose, survived in sorghum stalk debris on the soil surface for 18 mo. Germination of sclerotia decreased faster in sorghum stalk residues buried 10 and 20 cm in soil than in those kept on the soil surface. This suggests that sclerotia in sorghum debris on the soil surface may act as a primary source of inoculum for initiating anthracnose in the field.

Anthracnose, caused by *Colletotrichum graminicola* (Ces.) G.W. Wils. (syn. *C. sublineola* Henn. in Kab. & Bubák), is one of the most important diseases of sorghum (*Sorghum bicolor* (L.) Moench). This disease is especially severe in the warm humid areas of the world (2,7). Yield reductions of more than 50% have occurred in susceptible cultivars by the foliar phase of the disease (8).

C. graminicola survives in host plant residue and sorghum seeds as conidia and mycelia (15). Abundant production of sclerotia can be observed in dry stalks of susceptible sorghum cultivars at the end of the season. Although production of sclerotia by this pathogen is known (R. A. Frederiksen, *unpublished*), their longevity in sorghum stalk residues has not been determined. This work was carried out to determine the ability of *C. graminicola* to overseason in sorghum stalk residue in a noncropped soil area over two seasons.

Present address of first author: EMBRAPA, Centro Nacional de Pesquisa de Milho e Sorgo, Caixa Postal 151, Sete Lagoas 35700, Brazil.

Accepted for publication 26 April 1993.

MATERIALS AND METHODS

Germination of sclerotia in sorghum debris collected from the field. Sorghum stalks having a large number of sclerotia of *C. graminicola* (Fig. 1) were collected from the Field Laboratory of Texas A&M University located near College Station in Burleson County during August 1988 and 1989. Sclerotia were collected by scraping the stalk rind and vascular bundles with a scalpel and passing the scrapings through a 60-mesh sieve to separate sclerotia from stalk tissues. After surface sterilization for 3 min in a 0.5% NaClO solution, sclerotia were incubated under continuous light at 25 C on Cooke rose bengal agar (CRBA), on 1.8% water agar (WA), and in a dew chamber formed by moistened sterile filter paper in petri dishes (FP). Sclerotia were examined under a stereo light microscope 4 days later for the presence of acervuli, indicating germination. They were also measured several times by comparison to a grid of known size. A completely randomized design with three replications was used to evaluate sclerotial germination, and the experiment was repeated twice.

Survival of sclerotia in sorghum stalk residue. To determine the ability of *C. graminicola* sclerotia to survive under field conditions, samples of sorghum tissue were collected during August 1988 and 1989 from susceptible sorghum cultivars, as indicated by the presence of sclerotia. Several stalk pieces, 2–3 cm long, were placed in small nylon bags at depths of 0–2 (soil surface), 10, and 20 cm in three separate plots of a noncropped field area in September 1988 and 1989. This field plot was left fallow and uncultivated, and weeds were spot-sprayed with glyphosate (Roundup). The type of soil present in this area is a mixed alluvial, calcareous, Miller-Norwood-Pledger, clayey loam of basic pH. Samples were taken at 60-day intervals for 18 mo (September 1988 to March 1990

Fig. 1. Sclerotia of *Colletotrichum graminicola* in a sorghum stalk collected from the field at the end of the season (August 1989).

and September 1989 to March 1991). Ten sorghum stalk pieces were collected from each plot for examination. Sclerotia, recovered from sorghum stalks, were combined, treated as previously described, and plated in CRBA, approximately 100 per plate. Plates were incubated at 25 C under continuous light, and sclerotia were examined for evidence of germination 4 days later. Germination percentage was determined by counting the number of germinated sclerotia in 30 random fields of 25–30 sclerotia on one plate. Three plates were examined per replication per treatment. There were three replications for each 18-mo experiment.

Scanning electron microscopy of *C. graminicola* sclerotia. To observe germination, morphology, and structure of sclerotia of *C. graminicola*, samples obtained from infected sorghum, as described previously, were prepared for scanning electron microscopy in the Electron Microscopy Center in the Department of Biology of Texas A&M University, according to methods described by Brown and Wyllie (4).

Table 1. Average percent germination of *Colletotrichum graminicola* sclerotia on different culture media

Culture medium	Average percent germination[z]
Cooke rose bengal agar	80.2 a
Water agar	33.8 b
Filter paper	35.8 b

[z] Indicated by the presence of acervuli taken first from the average of three replications, and then the final average of two repetitions. Averages followed by the same letter are not significantly different according to Duncan's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

A significantly higher percentage of sclerotia germinated on CRBA than on WA or FP (Table 1). There was no significant difference for percent sclerotial germination between WA and FP.

Germination of sclerotia recovered from sorghum stalk residue was observed throughout the period of evaluation in both experiments. Since data from the two experiments were not statistically different, they were combined. The viability of sclerotia in stalk debris buried at 10 and 20 cm dropped more rapidly after the second month of the 18-mo test period than that of sclerotia in debris on the soil surface (Table 2, Fig. 2). Despite a decrease in germination on the soil surface, a significant proportion (26.5%) of sclerotia isolated from sorghum debris was still viable after 18 mo, whereas only 2.2 and 1.7% of sclerotia at 10 and 20 cm, respectively, germinated during the same period. Sclerotia germinated by producing acervuli with an abundance of falcate conidia (Fig. 3) and consisted of clusters of thick-walled cells with no defined zonations (Fig. 4). Measurements under the light microscope indicated that sclerotia were not uniform in size but varied from 125 to 440 μm.

A decrease in sclerotial germination on the soil surface has been reported for other species, although in some cases sclerotia below the soil surface survived better than those on the soil surface (1,10,12). It is believed that moisture deficit on the soil surface may cause a natural drying of sclerotia of *Sclerotium cepivorum* (Berk.) Whetzel (10). Rainfall or irrigation during summer months would provide the necessary moisture for the biological degradation of sclerotia (1). Pataky and Beute (12) considered that an interaction of temperature and moisture reduced viability of sclerotia of *Cylindrocladium crotalariae* (C.A. Loos) K.K. Bell & Sobus on the soil surface. The lower rate of degradation of sorghum debris on the soil surface probably promotes better sclerotial survival on the soil surface than in buried debris. Sorghum stalk residue may protect sclerotia from biological degradation caused by environmental fluctuations. This hypothesis was considered as a possible explanation for the survival of microsclerotia of *Macrophomina phaseolina* (Tassi) Goidanich in sorghum and corn stalk residues on the soil surface (5).

Survival of inoculum from sorghum in the form of sclerotia parallels survival of *C. graminicola* from maize. Vizvary and Warren (14) observed that conidia of *C. graminicola* attacking maize survive for only a few days, depending on the soil temperature and other environmental factors. Lipps (11) observed overwintering survival of *C. graminicola* in maize stalks in Ohio. *C. graminicola* survived longer in stalks left on the surface than in those buried. These observations supported the belief that minimum tillage practices increase the probability of anthracnose in production fields. Our studies focused on the survival of sclerotia of *C. graminicola* isolates attacking sorghum. These sclerotia survived for at least two noncropping seasons and survived best on the soil surface.

The fact that sclerotia of *C. graminicola* survived poorly in sorghum debris below the soil surface raises questions about possible factors influencing their degradation. Considering the previous hypothesis of protection provided by less

Table 2. Average percent germination of *Colletotrichum graminicola* sclerotia over time at different soil depths

Time (mo)	Average percent germination at soil depth[z]		
	0–2 cm	10 cm	20 cm
2	80.11 a	80.24 a	80.15 a
4	72.11 a	60.50 b	45.90 c
6	71.90 a	46.80 b	44.80 b
8	64.40 a	33.50 b	19.40 c
10	45.80 a	21.50 b	18.20 b
12	51.70 a	17.90 b	16.26 b
14	42.40 a	5.12 b	2.67 c
18	26.50 a	2.20 b	1.70 b

[z] Indicated by the presence of acervuli taken first from the average of three replications, and then the final average of two repetitions. Averages followed by the same letter in the same row are not significantly different according to the LSD test ($\alpha = 0.05$).

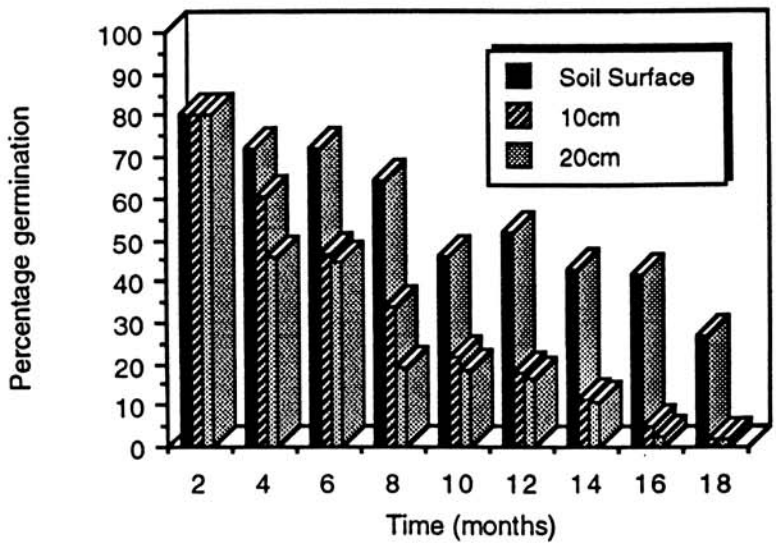

Fig. 2. Germination of sclerotia of *Colletotrichum graminicola* over time at three different soil depths.

degraded sorghum residues on the soil surface, it is reasonable to believe that faster decay of sorghum debris below the soil surface facilitates colonization of sclerotia by other microorganisms present in the soil microflora. It is also possible that at least a fraction of the nongerminating sclerotia is dormant. That sclerotia germinated better on CRBA than on WA and FP indicates a possible nutrient-dependent germination. A nutrient-dependent fungistasis was found to be responsible for the failure of microsclerotia of *Verticillium dahliae* Kleb. to germinate in soil (6). Also, germination of microsclerotia of *M. phaseolina* was demonstrated to be significantly stimulated by root exudates of jute and rice (3).

Sclerotia of *C. graminicola* in sorghum residues residing on the soil surface play an important role as a source of inoculum in the field. Indeed, in the Field Laboratory of Texas A&M University, the incidence of anthracnose is significantly higher in field plots with sorghum debris bearing sclerotia than in noninoculated plots (*unpublished*). Sclerotia have also been reported as a primary source of inoculum for other anthracnose fungi, i.e., *Microdochium panattonianum* (Berl.) Sutton, Galea, & Price in Galea, Price, & Sutton in lettuce (13) and *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore in soybean (9).

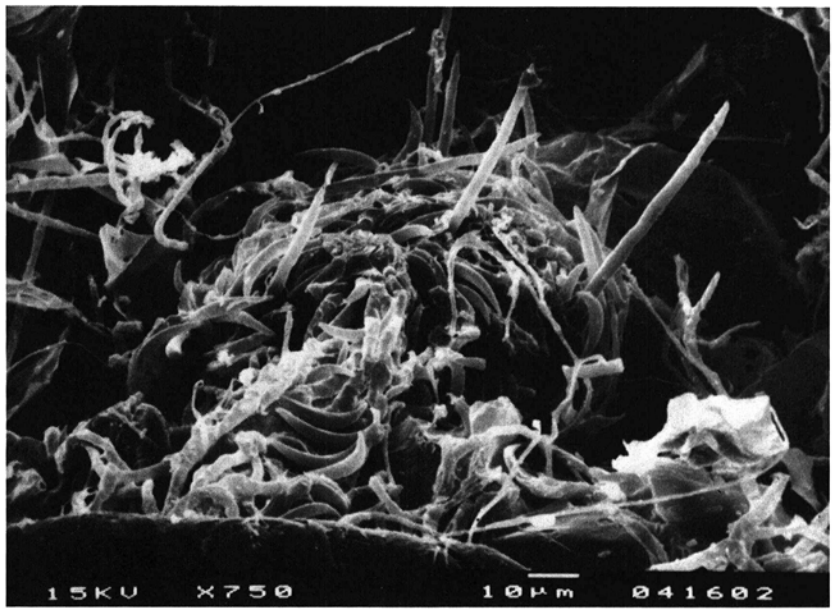

Fig. 3. Scanning electron micrograph of a germinated sclerotium of *Colletotrichum graminicola* showing an acervulus with falcate conidia.

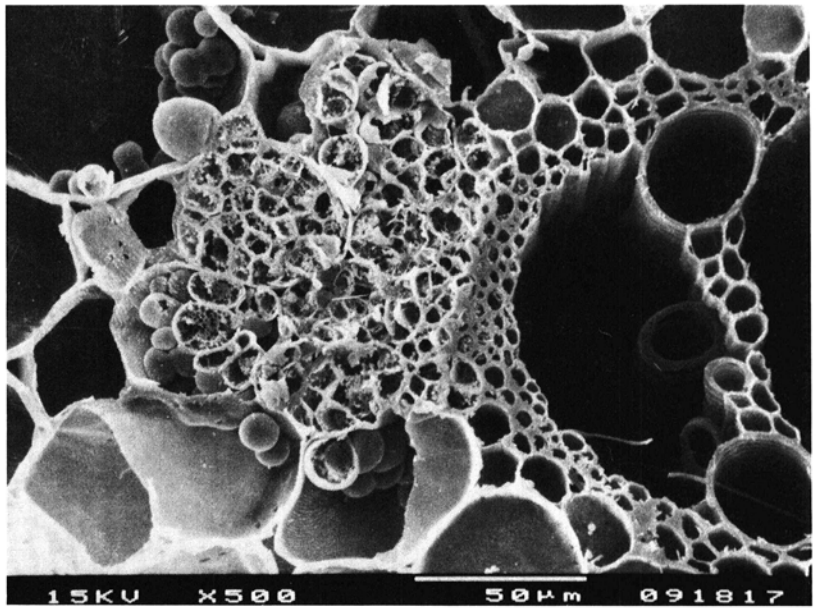

Fig. 4. Scanning electron micrograph of a transverse section of a sorghum stalk showing a sclerotium of *Colletotrichum graminicola* within the stalk tissue and to the left of a sorghum vascular bundle.

LITERATURE CITED

1. Adams, P. B. 1987. Effects of soil temperature, moisture, and depth on survival and activity of *Sclerotinia minor, Sclerotium cepivorum*, and *Sporidesmium sclerotivorum*. Plant Dis. 71:170-174.
2. Ali, M. E. K., and Warren, H. L. 1992. Anthracnose of sorghum. Pages 203-208 in: Sorghum and Millet Diseases: A Second World Review. W. A. J. de Milliano, R. A. Frederiksen, and G. D. Bengston, eds. ICRISAT, Patancheru, India.
3. Bhattacharya, M., and Samaddar, K. R. 1976. Epidemiological studies on jute diseases: Survival of *Macrophomina phaseoli* (Maubl.) Ashby in soil. Plant Soil 44:27-36.
4. Brown, M. F., and Wyllie, T. D. 1970. Ultrastructure of microsclerotia of *Verticillium albo-atrum*. Phytopathology 60:538-542.
5. Cook, G. E., Boosalis, M. G., Dunkle, L. D., and Odvody, G. N. 1973. Survival of *Macrophomina phaseoli* in corn and sorghum stalk residue. Plant Dis. Rep. 57:873-875.
6. Emmatty, D. A., and Green, R. J., Jr. 1969. Fungistasis and the behavior of the microsclerotia of *Verticillium albo-atrum* in soil. Phytopathology 59:1590-1595.
7. Frederiksen, R. A. 1984. Anthracnose stalk rot. Pages 37-42 in: Sorghum Root and Stalk Rots, a Critical Review. L. K. Mughogho, ed. ICRISAT, Patancheru, India.
8. Harris, H. B., Johnson, B. J., Dobson, J. W., Jr., and Luttrell, E. S. 1964. Evaluation of anthracnose on grain sorghum. Crop Sci. 4:460-462.
9. Khan, M., and Sinclair, J. B. 1991. Effect of soil temperature on infection of soybean roots by sclerotia-forming isolates of *Colletotrichum truncatum*. Plant Dis. 75:1282-1285.
10. Leggett, M. E., and Rahe, J. E. 1985. Factors affecting the survival of sclerotia of *Sclerotium cepivorum* in the Fraser Valley of British Columbia. Ann. Appl. Biol. 106:255-263.
11. Lipps, P. E. 1983. Survival of *Colletotrichum graminicola* in infested corn residues in Ohio. Plant Dis. 67:102-104.
12. Pataky, J. K., and Beute, M. K. 1983. Effects of inoculum burial, temperature, and soil moisture on survival of *Cylindrocladium crotalariae* microsclerotia in North Carolina. Plant Dis. 67:1379-1382.
13. Patterson, C. L., and Grogan, R. G. 1991. Role of microsclerotia as primary inoculum of *Microdochium panattonianum*, incitant of lettuce anthracnose. Plant Dis. 75:134-138.
14. Vizvary, M. A., and Warren, H. L. 1982. Survival of *Colletotrichum graminicola* in soil. Phytopathology 72:522-525.
15. Warren, H. L. 1986. Leaf anthracnose. Pages 10-11 in: Compendium of Sorghum Diseases. R. A. Frederiksen, ed. American Phytopathological Society, St. Paul, MN.