Overwintering Capacity of Ramulispora sorghi

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ABSTRACT

Ramulispora sorghi, causal agent of sooty stripe of sorghum, overwintered in Nebraska. A method for isolating *R. sorghi* sclerotia from soil was developed which involved wet screening, ammonium sulfate flotation, and centrifugation on a 70% (w/v) sucrose shelf. This isolation method was used in studies of survival under natural and artificial conditions in the field to demonstrate that sclerotia were the primary structure for survival of *R. sorghi*. Sporodochia also were important for fungal survival when sorghum production practices allowed infected leaf residue to remain on and above the soil surface throughout the winter. When such cultural practices are employed, maintenance of the connection between the sporodochium within leaf tissue and sclerotium on the leaf surface may be important to the survival of both fungal structures.

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Ramulispora sorghi (Ell. & Ev.) Olive and Lefebvre, causes sooty stripe, a foliar disease of Sorghum species (1). Since studies pertaining to the survival of *R. sorghi* were lacking (1), an investigation concerning the overwintering capacity of this organism was conducted. Evidence is presented from studies of winter survival which demonstrate that sclerotia are the primary structure for survival of *R. sorghi*.

MATERIALS AND METHODS.—Isolation of *Ramulispora sorghi* sclerotia from soil was achieved by a method involving wet-screening, ammonium sulfate flotation (3), and centrifugation on a 70% (w/v) sucrose shelf. Five to 70 g of air-dried field soil was ground to a fine powder using a mortar and pestle and deposited onto a 0.25-mm pore size (80-mesh) screen in tandem with a 0.047-mm pore size (300-mesh) screen. The screens were placed under running water and gently rubbed to disperse the larger soil particles. Material remaining on the 300-mesh screen was transferred to a 125-ml Erlenmeyer flask. Saturated ammonium sulfate was introduced to the bottom of the flask with a 50-ml burette and a small section of Tygon tubing. The ammonium sulfate and all floating material were collected in a second Erlenmeyer flask. The floating debris was collected on a Büchner funnel, resuspended in distilled water, and layered into two 30-ml centrifuge tubes containing 5 ml of 70% (w/v) sucrose overlayed with 5 ml of distilled water. The tubes were centrifuged in a swinging bucket rotor for 3 min at 2,000 *g*. Soil sedimented to the bottom of the tube enabling sclerotia to be collected from the sucrose-water interface (Fig. 1) and filtered on a Büchner funnel. The sclerotia of *R. sorghi*, distinguishable by size, shape, and rough wall characteristics, were either counted microscopically or surface sterilized and incubated on water agar. After 2 days, the sporogenic sclerotia of *R. sorghi* germinated and were identified and quantitated.

An alternate method for isolating sclerotia from soil was investigated. In this procedure, the ammonium sulfate flotation steps were omitted. Soil was wet-screened and material remaining on the 0.047-mm screen was resuspended, layered over sucrose, centrifuged, and treated as above.

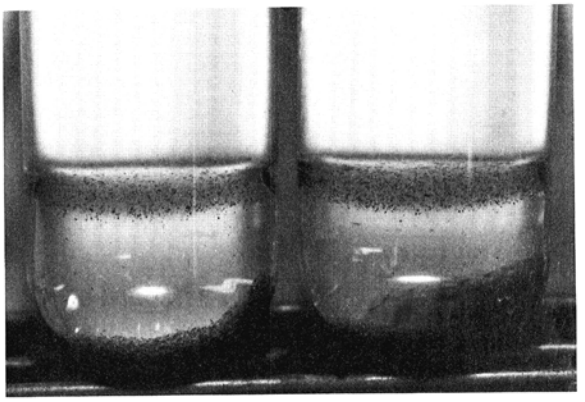

Fig. 1. Separation of sclerotia from soil by centrifugation for 3 min at 2,000 *g*. A sclerotial suspension was layered into two 30-ml centrifuge tubes containing 5 ml of sucrose (70%, w/v) and 5 ml of distilled water. After centrifugation, sclerotia remain at the sucrose-water interface, whereas soil sediments through the sucrose shelf.

To determine the ability and mode of overwintering of *R. sorghi*, studies of winter survival under natural and artificial conditions in the field were conducted at separate locations in southeastern Nebraska. The study of survival under natural conditions was conducted in a grain sorghum field in which a previous crop had had a high incidence of sooty stripe. Samples of field soil, and of infected leaf material on and above the soil surface, were collected monthly from November, 1971, until June, 1972. Infected leaf material was surface-sterilized with 1% NaOCl and placed on water agar to determine the presence of viable structures of *R. sorghi* (e.g., mycelium, sporodochia, or sclerotia). In addition, numbers of viable sclerotia per unit of soil were determined by the isolation procedure described above. The survival study under artificial conditions was conducted using leaf material infected with *R. sorghi*; leaves with intact sclerotia were placed into washed nylon mesh bags which were buried 0.61 m (2 ft) apart at soil depths of 0, 5.1, 10.2, 20.4, and 30.5 cm (0, 2, 4, 8, and 12 inches) or suspended above the

soil surface. Each treatment was replicated four times in a randomized block design. In separate plots, leaves were buried or suspended in November, 1971, and removed and evaluated for *R. sorghi* survival in June, 1972. Leaf material or leaf debris and soil in the bags were assayed for viable structures of *R. sorghi*.

RESULTS AND DISCUSSION.—Sclerotia were isolated from field soil to quantitatively determine the survival of *R. sorghi* sclerotia per unit of soil. Soil samples from 5 to 70 g were used to determine the most representative sample size for each of the two previously described methods of isolating sclerotia from soil. A sample size of 25 to 50 g of soil was optimal for the method involving ammonium sulfate flotation, whereas 5 g of soil was the optimal sample size for the alternate method (without ammonium sulfate flotation). For quantitative determinations, the ammonium sulfate flotation method was superior and resulted in less contamination by foreign particles. Approximately 100% of the *R. sorghi* sclerotia added to a soil sample were re-isolated; thus, the ammonium sulfate flotation method yielded quantities of *R. sorghi* sclerotia which were representative of the total number in a given soil sample. After treatment with saturated ammonium sulfate for 1 hr and 1% NaOCl for 1 min, 98% of the *R. sorghi* sclerotia germinated compared with 94% when treated with NaOCl only. Therefore, at the concns used and for the durations of treatment, NaOCl and ammonium sulfate were not detrimental to the germinability of *R. sorghi* sclerotia. Assays of field soil containing *R. sorghi* sclerotia yielded 180 sclerotia/g of which over 75% were sclerotia of *R. sorghi*. The remainder were sclerotia of other fungi and many germinated in these tests. When this method was used with soil containing sclerotia of fungi other than *R. sorghi*, approximately 48 sclerotia/g of soil were isolated. This method or a modification of it could be used to isolate sclerotia or other propagules from soil or other materials.

Viable mycelia of *R. sorghi* were not recovered from soil or infected leaves; therefore, survival of *R. sorghi* was determined by conidial production from sclerotia or sporodochia. Survival studies under natural conditions in the field indicated a general decline in the number of viable sclerotia in soil during the collection period (Table 1). Large proportions of sclerotia and sporodochia remained viable when associated with leaf tissue on or above the soil surface. Survival studies under artificial conditions in the field demonstrated that sclerotia, intact on leaf tissue, survived below, on, and above the soil surface, whereas sporodochia survived in leaf tissue on and above the soil surface but not below (Table 2). These studies suggest that sclerotia are the primary survival structures of *R. sorghi*, although sporodochia may be important for fungal survival if infected leaves are not destroyed by tillage practices or microbial activity.

A mature sclerotium of *R. sorghi* is entirely superficial on the leaf lesion but connected to a subepidermal sporodochium by a narrow column of fungal hyphae (2). Although these sclerotia were brittle and easily removed from lesions, large numbers remained on leaf tissue throughout the winter. Our studies indicated that, under certain field cultural practices, maintenance of the connection between the sporodochium and sclerotium could be important for survival of *R. sorghi*. Survival of sclerotia on and above the soil surface was two to three times greater than survival below the soil surface. All sclerotia on and above the soil surface were associated with sporodochia within leaf tissue since free sclerotia must have been incorporated into the soil. Below the soil surface, sclerotia were either free or attached to sporodochia.

Sclerotia of *R. sorghi* germinated through that portion of the sclerotial wall attached or previously attached to the subepidermal sporodochia. This is in direct contrast to the observations of Olive et al. (1) who suggested that germination occurs at the top of the sclerotium. The effect of the sclerotial-sporodochial attachment on the germination of either or both structures was determined. Treatments with distilled water resulted in limited germination of either the sporodochia or sclerotia, whether they were attached or free. Treatment with 1% NaOCl stimulated the germination of both structures, but no significant differences were noted between germination of those attached or free. The attachment between the sporodochium and the sclerotium may protect both

TABLE 1. Survival of *Ramulispora sorghi* in Nebraska under natural conditions[a]

Month of collection	Origin of sclerotia		
	Soil	Leaves on soil surface	Leaves above soil surface
	% of sclerotia germinating		
1971 November	80	98	98
December	---	---	---
1972 January	78	93	95
February	31	98	90
March	55	78	72
April[b]	34	96	96
May[b]	38	90	90
June[b]	27	---	---

[a] The study was conducted in a sorghum field previously having a high incidence of sooty stripe. Samples of field soil and of leaf material were evaluated monthly.
[b] Material collected from an adjacent field.

TABLE 2. Survival of *Ramulispora sorghi* in Nebraska under artificial conditions[a]

Incubation depth in soil (cm)	Germination of sclerotia[b] (%)		
	Rep I	Rep II	Average
30.5	46	57	52
20.3	28	44	36
10.2	23	67	45
5.1	32	28	30
On soil surface	59	41	50
Above soil surface	64	78	71

[a] Infected leaves with intact sclerotia were placed in washed nylon mesh bags and buried or suspended above the soil surface in November, 1971. Leaf material or leaf residue and soil in the bags were assayed for the presence of viable *R. sorghi* mycelium, sporodochia, or sclerotia in June, 1972.
[b] A control sample of sclerotia intact on leaf tissue was maintained at 4 C for the duration of the experiment. The germination of these sclerotia was 90%.

structures from microbial attack and from adverse environmental factors, but it appeared to have little effect on the capacity of either structure to germinate.

Sooty stripe of sorghum is a common disease in the southern United States that is spread in the field by wind and rain splash (2). Movement of spores is probably limited so that occurrence and establishment of the disease in more northern areas of the United States depends on the proximity of inoculum. Thus, winter survival is of paramount importance. Present studies demonstrated that *R. sorghi* did survive the winter in Nebraska. Sclerotia were the primary structure for survival of the fungus, but under certain field cultural conditions sporodochia may be important for fungal survival. Although conidia produced by germinating sclerotia and sporodochia have not been observed in the field, they are apparently the initial inoculum for infection in the spring.

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