Effects of Soil Moisture, Temperature, and Field Environment on Survival of *Sclerotium rolfsii* in Alabama and North Carolina

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ABSTRACT

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Sclerotia of *Sclerotium rolfsii* survived in high numbers during the months of November 1977 through August 1978 in peanut fields in North Carolina. Viability of sclerotia produced in field soil ranged from 56 to 73% in field microplots after 8–10 mo of incubation. In growth chamber tests, survival of sclerotia was less at temperatures above 20 C in moist field soil than at 20 C or less. No adverse effect of temperature on sclerotial survival was observed in dry field soil. Mycelia of *S. rolfsii* rapidly died in moist field soil, but they survived for at least 6 mo in dry soil. *S. rolfsii* grew on peanut stems buried in field soil and produced new sclerotia. The presence of organic substrate and/or associated microorganisms was detrimental to survival of adjacent sclerotia.

Additional key words: Arachis hypogaea, plant residue, southern stem rot.

There has been debate over factors influencing the survivability of sclerotia of *Sclerotium rolfsii* Sacc. (8). Survival of sclerotia produced in sterile culture was influenced primarily by moisture and temperature; sclerotia of *S. rolfsii* remained viable for 2–5 yr when kept dry (1). As recently as 1971, Roy (14) found that germinability of sclerotia of *Corticium sasaki* (Shirai) Matsumoto produced in sterile culture was 50–100% for 4-yr-old sclerotia stored at room temperature in petri dishes containing moistened field soil, and he suggested rotations having >4 yr without plants susceptible to *C. sasaki* to avoid damage to host crops. Although Williams and Western (16) report survival of sclerotia of *Sclerotium delphinii* for 2 yr in field soil, Javed and Coley-Smith (8) found that survival of sclerotia of that pathogen buried at 7.5 and 15.0 cm in moist soil decreased markedly after 6–9 mo. Other investigators report that only 60% of sclerotia of *S. rolfsii* buried 10.2 cm deep in field soil was viable after 60 days and none buried 15.3 cm deep germinated after 45 days (6). Sclerotia grown in sterile culture, dried for short periods, then remoistened survived <2 wk in moist field soil and exuded large quantities of sugars and amino acids which were thought to promote microbial breakdown of sclerotia in soil (15). Linderman and Gilbert (10), working with

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sclerotia grown in sterile culture, proposed that sclerotial leachates induced or enhanced a mycosphere effect that increased the capacity of soil to inhibit mycelial growth from sclerotia. They suggested that *S. rolfsii* contributed to its own inhibition in soil by inducing changes in soil the microflora, the metabolites of which restricted mycelial growth from sclerotia. In a subsequent report, Linderman and Gilbert (11) demonstrated that sclerotia produced in nonsterile field soil differ physiologically from those grown in sterile culture. This was further substantiated by Beute and Rodríguez-Kabana (3,4) who showed that germination of field-grown sclerotia was induced primarily by the presence of stimulatory factors emanating from senescent or dead plant tissues.

Cultures of *S. rolfsii* grown on autoclaved wheat or oat seeds can be dried and stored for many months with no apparent loss in viability (7). Epps et al (7) reported, however, that mycelium of *S. rolfsii* in infested seed was not viable after 1 mo at 50% RH and 30 C. Infested seed stored at 12 or 25% RH for 1 yr at 7–8 C produced vigorous colonies of *S. rolfsii* when placed on nutrient agar.

Despite the inability of investigators to agree on the survival characteristics of *S. rolfsii*, the fungus persists in field soil for many seasons, through wet and dry periods, and through nonhost croppings. For 4 yr (1932–1936), Leach and Davey (9) followed inoculum densities of *S. rolfsii* from successive soil analyses in eight fields. They showed that numbers of sclerotia were influenced primarily by the crops grown and that relative inoculum density in soil could be used to predict disease incidence in sugar beet production.

The purpose of this study was to determine survival capacity (viability) of sclerotia found in peanut soils in North Carolina and Alabama, to compare survival of mycelium and sclerotia of *S. rolfsii* at 15–35 C in field soil maintained at high and low moisture levels, and to evaluate the ability of *S. rolfsii* to produce additional sclerotia in field soil when organic debris was present.

MATERIALS AND METHODS

Inoculum production. The culture of *S. rolfsii* used in Alabama tests was isolated from infected peanut (*Arachis hypogea* 'Florunner') collected at the Wiregrass Substation, Headland, AL. The culture of *S. rolfsii* used in North Carolina was isolated from *A. hypogea* 'Florigiant' collected in Bertie County, NC. Isolates were grown on potato-dextrose agar and transferred either to autoclaved oat grains or peanut stem pieces (2 cm long) (12). After 8 days of growth at 27 C, the oats or peanut stems were air-dried and stored in the dark at 4 C. To produce sclerotia, oat inoculum was spread on the flattened surface of moist (60% field capacity) sandy loam field soil (4). After 1 wk in the greenhouse, mature sclerotia were collected, air-dried, and stored at room temperature (27 C) until used.

Growth chamber tests. Effects of temperature and soil moisture on *S. rolfsii* survival as sclerotia or mycelium were studied simultaneously in growth chambers (Freas 815, Precision Scientific Co., Chicago, IL 60647). Five growth chambers (set at 15,20,25,30, or 35 C) were used with two soil moisture levels. Sandy loam field soil (180 g) was added to 6.5-cm-diameter plastic cups. Soil moisture was adjusted to 1.7% H_2O (v/w) for the dry soil treatment and 5.8% H_2O (v/w) for the moist soil treatment (−15 and −1/3 bars tension, respectively, according to data supplied by C. B. Elkins, Agronomy Department, Auburn University, Auburn, AL). Cups were placed in plastic bags and closed with a rubber band prior to placement in growth chambers. Sufficient H_2O was added periodically to each cup to replace moisture lost during incubation. Average H_2O added monthly per pot at each temperature was: 15 C, 4.0 ml; 20 C, 5.8 ml; 25 C, 7.5 ml; 30 C, 8.0 ml; and 35 C, 10.5 ml. Ten sclerotia were placed in small nylon bags (2×4 cm) and inserted into the soil 5–6 cm deep. Ten sclerotia were also placed on the soil surface. In other treatments, 10 oat grains infested with *S. rolfsii* were either buried in soil in nylon bags or placed on the soil surface.

Four cups from each treatment (temperature × moisture × propagule type) were removed from each of the chambers at 1, 2, 3, 4, 5, and 6 mo after initiation of the test. Propagules were placed on medium selective for *S. rolfsii* to test for viability (2). Growth determinations were made after 48 hr of incubation at room temperature (27 C).

Microplot field tests. Plastic PVC pipe (10.2 cm in diameter) was cut into 15.3-cm lengths and positioned 12 cm deep in field plots at the Auburn University Agronomy Farm, Auburn, AL. The soil was a Chesterfield sandy loam that had been planted to peanut for the preceding 2 yr. Soil within the pipes was removed to a depth of 5 cm and a fine-mesh nylon cloth bag was placed in the pipe. Various combinations of sclerotia and/or peanut stems were placed on the cloth. A second cloth was placed over the amendments and soil was gently packed to the original soil line. The following treatments were established: 10 sclerotia, 10 sclerotia and six sterile peanut stem sections (2 cm long), 10 sclerotia and six nonsterile peanut stems, six peanut stems infected with *S. rolfsii*, and six nonsterile healthy peanut stems. Six replications of each treatment were examined at each assay date. The test was begun on 27 October 1977 and sampled on third week of the succeeding 7 mo (November, December, January, March, April, June, and August). Sclerotia and/or peanut stems were placed on medium selective for *S. rolfsii* (2) and growth was determined after 48 hr.

A similar test was conducted in Norfolk sandy loam at North Carolina State University, Raleigh. In this test, however, only sclerotia and *S. rolfsii*-infected peanut stem treatments were studied.

Field assay. Field A (in Northampton County, NC) and Field B (in Bertie County, NC) were selected for studies of survival of *S. rolfsii*. Both fields had a high incidence of southern stem rot in peanuts in 1977. Composite soil samples (30–40 cores 24 mm in diameter × 15.3 cm long) taken randomly from three areas (each ~1 ha) in each field were elutriated (5) to separate propagules from soil. Sclerotia and plant debris collected on 850-μm (20-mesh) sieves were placed on *S. rolfsii*-selective medium (2) and growth was determined after 48 hr. Soil samples were obtained from fallow areas in each field at monthly intervals (November–May) beginning approximately 1 mo after peanut harvest in North Carolina.

Influence of buried peanut stems on sclerotial populations. Because newly formed sclerotia of *S. rolfsii* were commonly found in both growth chamber and microplot tests, additional microplots were established at the Auburn University Agronomy Farm to determine the role of buried peanut stems in reproduction of *S. rolfsii* in the field. On 20 December, 1977, 10 sclerotia were buried 5–6 cm deep in 21 plots with and without six peanut stems. On 17 February 1978 (2 mo after sclerotia were added) six peanut stems were added to the 21 plots not previously receiving stems. In April, 1978, soil (containing sclerotia and stems) was removed to a 7–8 cm depth from plots and assayed for *S. rolfsii*. Viable propagules were detected by using a newly developed technique wherein soil is wetted with methanol in water (1:100, v/v) to promote germination and identification of *S. rolfsii* in intact soil samples (13). Determination of viable propagules was made after 3 days of incubation.

RESULTS

Growth chamber tests. An average of 46 and 6% of oat grains yielded *S. rolfsii* on selective medium following incubation for 1 and 2 mo, respectively, on the surface of soil maintained at −1/3 bar moisture tension; comparable figures for buried oat grains were 33 and 0%, respectively. No temperature effect was observed. Survival of *S. rolfsii* on oat grains in the low moisture treatment (−15 bars) at all temperatures was 100% after 2 mo. *S. rolfsii* could not be recovered from buried oat grains at 25 C after 3 mo. However, 28% of buried oat grains produced *S. rolfsii* after 6 mo when incubated at 15 and 35 C in dry soil. Survival of mycelia was greater at the soil surface (34%) than in those that were buried (22%).

Ninety-four percent of sclerotia in the low-moisture treatment were viable after 6 mo over all temperatures (Fig. 1). In the high-moisture treatment, however, survival was greater ($P = 0.05$) at 15 and 20 C than at 25, 30, and 35 C after 2–6 mo. An average of 97% of sclerotia were viable after 6 mo at 15 and 20 C (Fig. 2). Only 66% of sclerotia were viable at 25–35 C. Survival of sclerotia was

consistently greater at the soil surface (83%) than in those that were buried (74%).

Microplot field tests. Survival of sclerotia of *S. rolfsii* buried in nylon mesh bags without added organic substrate was 73% after 10 mo in Alabama. The proportion of sclerotia which remained viable was reduced ($P = 0.05$) to 34% after 10 mo in the presence of peanut stems (both sterile and nonsterile stems). No sclerotia were detected in plots established with peanut stems alone. In a similar test in North Carolina, 56% of the sclerotia buried in nylon mesh bags without added organic substrate were viable after 8 mo (Fig. 3). *S. rolfsii* was recovered on selective medium from infested peanut stems buried in nylon mesh bags after 3 mo (March) in North Carolina. However, *S. rolfsii* could not be recovered from buried peanut stems in Alabama after 2 mo (December).

Viability of buried sclerotia was reduced to 73% in plots without peanut stems and to 43% in the presence of peanut stems in the Alabama test. However, the total number of sclerotia present in microplots after 10 mo averaged 28% more in plots that received peanut stems. In a separate test in the same field plots, burial of sclerotia for 4 mo in the presence of peanut stems resulted in recovery of 48% more sclerotia (April) than from plots in which only sclerotia were buried. Incorporation of peanut stems into microplots 2 mo (February) after burial of sclerotia resulted in recovery of 47% more sclerotia from plots after an additional incubation of 2 mo (April) than from plots in which only sclerotia were buried.

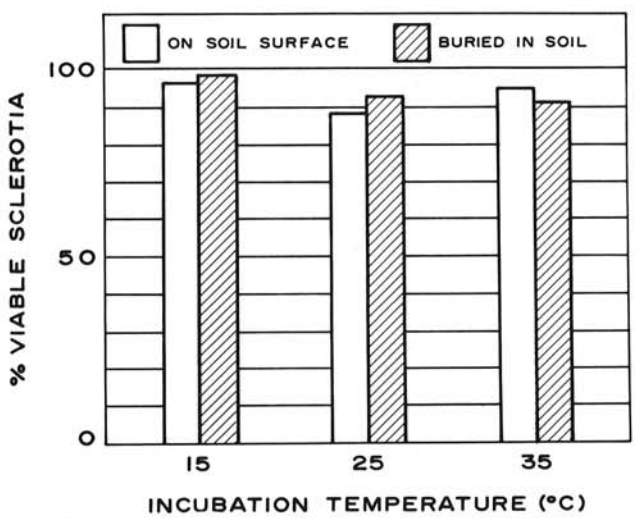

Fig. 1. Viability of sclerotia of *Sclerotium rolfsii* in soil with low moisture (−15 bars matric potential) after 6 mo at three temperatures.

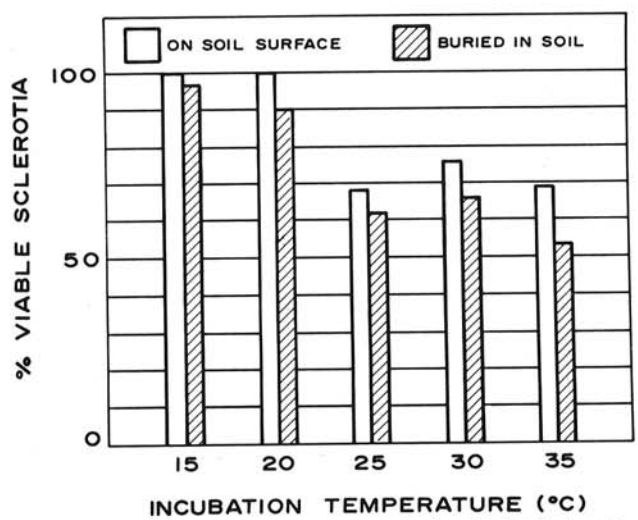

Fig. 2. Viability of sclerotia of *Sclerotium rolfsii* in soil with high moisture soil (−1/3 bar matric potential) after 6 mo at five temperatures.

North Carolina field assays. Decreasing recovery of *S. rolfsii* was noted from November through March, 1978, when peanut stem tissue from the 1977 crop (tissue selected on the basis of damage produced by *S. rolfsii*) was incubated on selective medium (Fig. 4). The proportion of sclerotia of *S. rolfsii* still viable was 73% 8 mo after harvest of the original peanut crop. Survival of sclerotia and mycelium in debris was essentially the same in both fields assayed. Viability of sclerotial populations appeared to decrease slightly as the soil temperatures increased (February–April) and then remained stable (Fig. 4) with a 27% decrease after 8 mo.

DISCUSSION

As suggested by Javed and Coley-Smith (8), survivability of sclerotia cannot be adequately evaluated until comparisons are made using many isolates of the fungus. In this study, large numbers of sclerotia (produced in field soil) from two isolates of *S. rolfsii* survived during all months in peanut fields of Alabama and North Carolina. Viability of sclerotia ranged from 56 to 73% of original population densities when sclerotia were recovered from microplots after 8–10 mo of incubation (November–August) in Alabama and North Carolina. Similarly, viability of recovered sclerotia (indigenous populations) from two commercial peanut

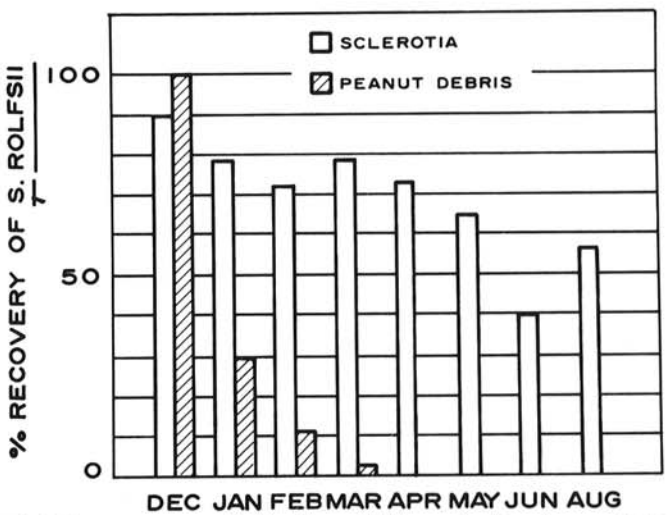

Fig. 3. Percent recovery of *Sclerotium rolfsii* from sclerotia and peanut debris overwintering in North Carolina field microplots from December 1977 to August 1978. Sixty sclerotia or pieces of debris were assayed on each date.

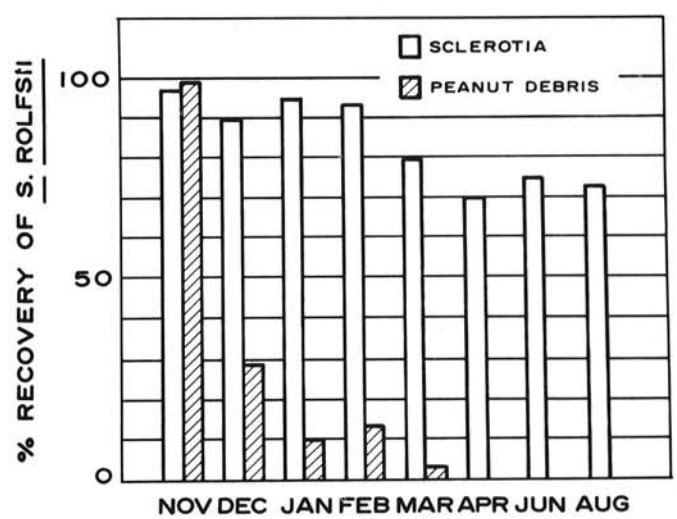

Fig. 4. Percent recovery of *Sclerotium rolfsii* from sclerotia and peanut debris overwintering in two North Carolina peanut fields (1977–1978). Similar data from two farms were combined. Sixty sclerotia or pieces of debris from each field were assayed each date.

fields in North Carolina was 73% 10 mo (August) after harvest. Percentages of sclerotial viability declined most during spring and early summer as temperature increased in both states.

Data from growth chamber tests suggest that temperature and moisture factors may interact in survival of *S. rolfsii* under field soil conditions. Under the high soil moisture regime, survival of sclerotia (in field soil) was significantly lower when temperatures were maintained above 20 C than at or below 20 C. No adverse effect of temperature on survival of sclerotia was observed in any of the low-moisture treatments. Burial (5–6 cm) decreased the viability of sclerotia compared to those on the soil surface. Mycelium of *S. rolfsii* rapidly died in the presence of high moisture at all temperatures tested in this study. However, *S. rolfsii* mycelium was viable for at least 6 mo at 15 and 35 C in dry soil.

S. rolfsii in peanut stems buried in field soil produced additional new sclerotia. It appears, however, that the presence of organic substrate and/or associated microorganisms was detrimental to survival of sclerotia since percent viability of sclerotia was less even though total numbers of sclerotia had increased. Although viability of sclerotia of *S. rolfsii* decreased over time, there was high survival of sclerotia on or near the soil surface in peanut fields of Alabama and North Carolina. Addition of nonhost plant debris to soil through normal tillage during crop rotation may hasten the degradation of viable sclerotia.

Discrepancies between results of these tests and previous investigations may result from the type of sclerotia used in tests. The survival potential of *S. rolfsii* is not realistically reflected when dried sclerotia grown in sterile-culture are used in laboratory tests. As previously noted (4), the results of studies involving sclerotia grown in sterile-culture and exposed to field soil are also misleading. Although we believe the results of this study more adequately reflect the survival potential of *S. rolfsii*, more work is needed to evaluate the effects of variable soil and environmental conditions.

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