Effect of Wetting and the Presence of Peanut Tissues on Germination of Sclerotia of *Sclerotium rolfsii* Produced in Soil

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ABSTRACT

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Wetting of *Sclerotium rolfsii* sclerotia produced in peanut field soil did not enhance germination unless other stimulants also were present. Field-produced sclerotia germinated and *S. rolfsii* grew profusely in the presence of remoistened dried green peanut stems and leaves, but not in the presence of partially decomposed peanut debris from the field. Dried green leaves from peanut cultivars Florigiant, Florunner, NC 2, NC 3033, and a Spanish selection (C_2) all stimulated equal amounts of sclerotial germination.

Additional key words: Arachis hypogaea, southern stem rot.

Southern stem rot of peanut (which is caused by *Sclerotium rolfsii* Sacc.) has been reported from all peanut (*Arachis hypogaea* L.)-growing areas of the world and is of major importance in peanut culture (4). The favorable influence of high soil moisture on disease development has been noted many times and serious outbreaks often are associated with unusually wet seasons (1). A recent report indicated that drying and subsequent rewetting of sclerotia of *S. rolfsii* enhanced both germination and microbial breakdown of sclerotia in soil (7,8). Even though drought and heavy rains occur regularly in fields in the southeastern USA, the disease continues to be of major importance. Volatile components in a distillate of

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alfalfa hay also have been reported to stimulate growth of *S. rolfsii* on agar and in soil (5). However, these tests have been conducted with sclerotia produced in sterile cultures. Recently it was suggested (6) that sclerotia produced in soil do not physiologically resemble those produced in culture.

These studies were conducted to evaluate the role of wetting or leaching of dried sclerotia by rain in initiating sclerotial germination and to determine the effects of other factors, such as the presence of peanut tissues or crop debris, on the germination of sclerotia.

MATERIALS AND METHODS

Production of sclerotia. The isolate of *S. rolfsii* used in these studies was from infected 'Florunner' peanuts collected at the Wiregrass Substation at Headland, Alabama. The culture was grown on potato-dextrose agar (PDA) and transferred to autoclaved oat grains in 500-ml flasks. After 8 days of incubation, the fungus-infested oats were air-dried and kept in the dark at 4 C. Oat inoculum was spread on the flattened surface of moist (approximately 60% field capacity) sandy loam from a field planted to soybeans the previous season; 35 × 50 cm trays were filled with soil to a depth of 8 cm. Oat grains were added at the rate of one per square centimeter and trays were covered with a layer of Saran® (Dow Chemical Co., Midland, MI 48640) wrap to minimize moisture loss. After 1 wk in the greenhouse, mature sclerotia were collected, air-dried in the laboratory (in equilibrium with 70–95% RH of ambient air), and kept in a vial at room temperature (27 C) until used. Other sclerotia were grown in sterile culture on PDA in 9-cm-diameter petri plates, air-dried, and kept in similar vials.

Wetting of sclerotia. An apparatus was constructed to simulate leaching of dry sclerotia with rain. A 250-ml cylinder was suspended over a reservoir (7.5-cm diameter × 15 cm) containing rubber plugs at both ends (Fig. 1). Five capillary tubes arranged equidistant from each other and the periphery of the cylinder were inserted into the lower plug. Tygon tubing connected the top and bottom of the cylinder to the reservoir. A third tube from the reservoir was connected to a capillary tube submerged in 3 cm of water in a glass tube (2.5 × 10 cm). A second rubber tubing attached to the glass tube was fitted with a pinch clamp to control air flow through the tube into the reservoir and thus, to adjust the number of drops delivered per hour from the five capillary tubes. Ten sclerotia were placed on glass filters (Reeve-Angel Co., Clifton, NJ 07012) suspended on a Fiberglas screen in 7.5-cm-diameter cylinders approximately 10 cm below the reservoir. Sclerotia were either leached for 1 hr in this apparatus or were soaked for a similar time in equivalent quantities of water (110 ml or 220 ml equivalent to 25 or 50 mm of rain per hour, respectively). Leached, soaked, or dry sclerotia were implanted in Norfolk sandy loam in 9- or 14-cm-diameter petri plates containing 1 g BaO_2 (to supply O_2 and remove CO_2) plus 2 ml of water in a small vial placed in the center of the plate (3). Percentage germination and measurement of mycelial growth were determined after 48–72 hr.

Evaluation of peanut tissue as a stimulant of sclerotial germination. Dried green peanut stems, leaves, and finely ground peanut tissues as well as partially decomposed peanut tissues were moistened and tested for stimulatory effects on sclerotial germination and mycelial growth on Norfolk sandy loam soil. Partially decomposed peanut stems and leaves (1977 crop) were collected from the Auburn University Research Farm, Auburn, Alabama, in January 1978. In the sclerotial wetting tests either a 2-cm length of dried green peanut stem (about 30 mg) or 100 mg of dried finely-ground green peanut tissue in a small vial were placed in the petri dishes to test for effects on germination by volatile and water-soluble stimulants. Other sclerotia were assayed routinely in soil plates prepared as follows: plastic rings (5.5 cm in diameter × 1 cm high) were glued with a small amount of silicone rubber to the border of circles of nylon screen (mesh = 1 mm) to form small sieves. The sieves were allowed to stand for 24 hr, were filled with moist sandy loam field soil, and the surface was smoothed (Fig. 2). Ten sclerotia were implanted on the soil surface of each plate by arranging them radially 1 cm from the periphery of the plate. Seven plates with sclerotia were placed on a wire screen (1.5-cm mesh) suspended 5 cm above the bottom of 24-cm-diameter glass desiccators. A 5-cm-diameter petri dish containing 5 g of BaO_2 and 10 ml of water was placed on the wire screen. In some tests, dried green peanut stems (2 cm long) were implanted in moist sandy loam or washed sea sand (681 g) (Fisher Scientific, Pittsburgh, PA, 15219) in the bottom of the desiccators. In other tests, various

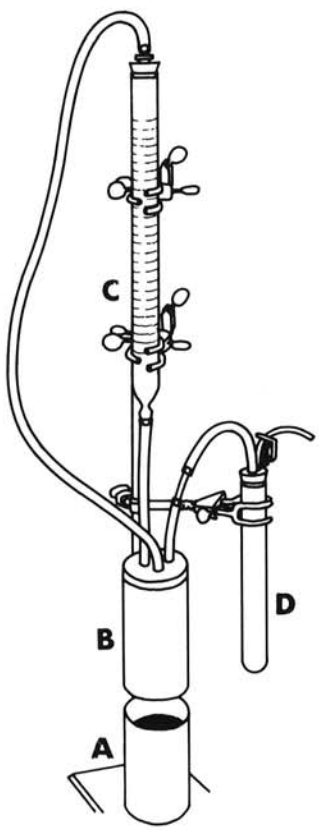

Fig. 1. Leaching apparatus for testing germination of sclerotia of *Sclerotium rolfsii*. Legend: A = fiberglass filter supporting sclerotia; B = reservoir containing five capillary tubes in lower side; C = 250-ml graduated cylinder; and D = airflow regulator tube with pinch clamp.

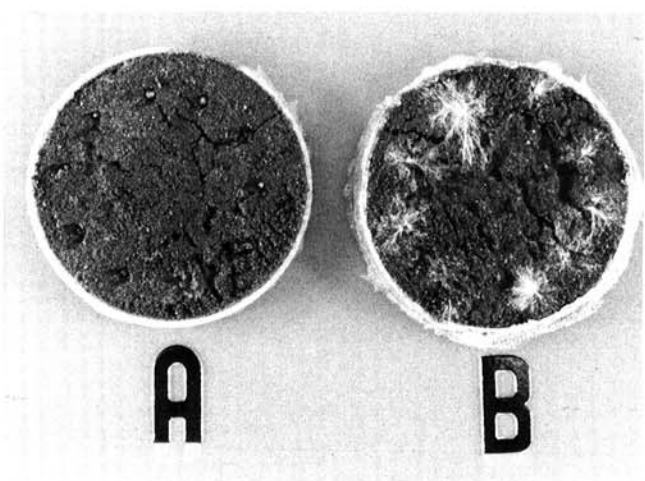

Fig. 2. Soil plates (5.5 cm in diameter) containing 10 sclerotia of *Sclerotium rolfsii* implanted on the soil surface. Plate A, sclerotia incubated 48 hr in desiccators without amendments. Plate B, sclerotia incubated 48 hr in desiccators that received 2 g of dried peanut hay in a petri dish in the bottom.

quantities of dried, finely ground, remoistened green tissues from peanut cultivars Florigiant, Florunner, NC 2, NC 3033, and a Spanish selection (C_2) were placed in the bottom of the desiccators in 9-cm-diameter petri plates. After 48 hr, the number of germinating sclerotia was counted and the extent of growth was estimated according to a scale of 1 to 4, in which 4 represented growth obtained in the control treatment (amended with 2 g Florunner hay) and 1 represented no growth.

RESULTS

Effect of wetting sclerotia. In our first test with air-dried sclerotia produced in soil, no differences in germination were noted between treatments in which sclerotia had been soaked in 110 ml of deionized water for 1, 2, 4, or 9 hr prior to being placed on moist soil, or in those leached with 12.5, 25, or 50 ml of water during a 1-hr period (Table 1). Although less than 18% of these sclerotia germinated on the soil surface, all sclerotia germinated when placed on a medium selective for *S. rolfsii* (2). In contrast, 60–80% of air-dried sclerotia grown in sterile culture germinated regardless of whether they were soaked in water, leached with 25 or 50 ml of water for 1 hr, or placed dry on moist soil.

Placement of 0.1 g of moistened peanut hay in a vial in the center of the plates with sclerotia eliminated the difference in germination between sclerotia produced in soil and sclerotia produced in sterile culture (Table 2). Germination of sclerotia which were implanted dry was lower ($P = 0.05$) than that of sclerotia that were wetted by soaking or leaching (before placement on soil) either in plates that received hay amendments, or in plates receiving sterile-culture sclerotia.

Stimulation of sclerotia by peanut stems. Germination of soil-produced sclerotia was increased ($P = 0.01$) 4.7-fold by placement of 2 g of dried green peanut stems (2 cm long) on moist soil in the bottom of 24-cm-diameter desiccators as compared to similarly treated sclerotia without amendments (Table 3). Germination was consistently higher on soil plates than on Fiberglas filters in all treatments. Growth of *S. rolfsii* was increased ($P = 0.01$) on both soil plates and glass filters with peanut amendments.

Sea sand was used in the bottoms of certain desiccators to eliminate the possibility that stimulation from indigenous soil microflora or fauna might interfere with stimulants that originate from peanut tissue. Germination was higher ($P = 0.05$) on both glass filters and on soil plates in these treatments than in desiccators

TABLE 1. Effect of prewetting on germination of sclerotia of *Sclerotium rolfsii*

Treatment[y]	Germination[z] (%)	
	Soil sclerotia	Culture sclerotia
None	7 a	60 b
Soaked	18 a	80 b
25 mm (leached)	13 a	67 b
50 mm (leached)	17 a	80 b

[y] Sclerotia were subjected to a leaching regime of 25 or 50 mm water per hour for 1 hr, or soaked in 110 ml of water for 1 hr.
[z] Numbers are the average of three replications with 10 sclerotia per replicate. Numbers followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Effect of incorporation of peanut hay on germination of sclerotia of *Sclerotium rolfsii* with or without soaking or leaching of sclerotia

Leaching treatment[x]	Germination[y] (%)		
	Soil sclerotia	Soil sclerotia + peanut hay[z]	Culture sclerotia
Dry	7 a	20 b	23 b
Soaked	10 a	47 c	67 c
25 mm (leached)	7 a	53 c	57 c
50 mm (leached)	3 a	40 c	53 c
Average	7	40	50

[x] Numbers are the average percent germination of three replications of 10 sclerotia per replicate.
[y] Numbers followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.
[z] Remoistened, dried green peanut tissue (0.1 g/plate) was added in a vial in center of each plate.

TABLE 3. Effect of volatile compounds from peanut stems on germination of sclerotia of *Sclerotium rolfsii*

Desiccator bottom[x]	Amendment	Assay substrate	Germination[y] (%)	Growth index[z]
Field soil	Peanut stem (2g)	Sandy loam	76	3.4
		Glass filter	59	3.2
	No Hay	Sandy loam	20	2.1
		Glass filter	9	2.1
Sea sand	Peanut stem (2g)	Sandy loam	94	3.8
		Glass filter	73	2.8
	No Hay	Sandy loam	46	2.0
		Glass filter	13	2.2
LSD ($P = 0.05$)			8.9	0.3

[x] Each 24-cm-diameter desiccator received 681 g of moistened field soil or sea sand and dried green peanut stems (2 cm long) were implanted in the appropriate treatments.
[y] Seven soil plates were used per desiccator with 10 sclerotia per plate. Five grams of BaO_2 plus 10 ml of water in vials were added to each dessicator.
[z] Index is average of rating for all germinated sclerotia after 48 hr according to a scale of 1 to 4, in which 4 represented maximal growth and 2 represented germination, but little or no mycelial extension.

TABLE 4. Effect of incorporation of dried leaves of several peanut cultivars on germination of sclerotia of *Sclerotium rolfsii*

Cultivar	Amendment quantity (g)[x]	Germination[y] (%)	Growth index[z]
Spanish (C_2)	0.40	91 ab	2.3 D
	0.28	96 ab	2.4 CD
	0.05	63 c	2.1 E
	0.028	37 de	2.1 E
Florunner	0.50	97 ab	2.5 C
	0.28	87 ab	2.1 E
	0.05	64 c	2.0 F
	0.028	41 de	2.0 F
Florigiant	0.50	99 a	3.6 A
	0.28	91 ab	3.2 B
	0.05	67 c	2.2 DE
	0.028	46 d	2.0 F
NC 3033	0.50	90 ab	3.2 B
	0.28	83 b	3.0 BC
	0.05	61 c	2.1 E
	0.028	44 de	2.0 F
NC 2	0.50	97 ab	3.7 A
	0.28	94 ab	3.1 BC
	0.05	67 c	2.2 DE
	0.028	40 de	2.0 F
Check	...	34 e	2.0 F

[x] Moistened peanut tissues were placed in 9-cm-diameter petri plates in the bottom of 24-cm-diameter desiccators. Five grams of BaO_2 and 10 ml water in vials were added to each desiccator.
[y] Seven soil plates were used per desiccator with 10 sclerotia per plate. Numbers followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.
[z] Index is average of rating for all germinated sclerotia after 48 hr according to a scale of 1 to 4, in which 4 represented maximal growth and 2 represented germination, but little or no mycelial extention.

with field soil in the bottom. Growth of *S. rolfsii* was greater on soil plates but less on glass filters when peanut amendments were placed in sea sand when compared to field soil in desiccator bottoms. As with the field soil treatments, germination and growth of *S. rolfsii* was low when no peanut amendments were used.

Effect of peanut cultivars. When dried green peanut leaves were used as amendments as previously described for peanut stems, germination of soil-produced sclerotia and growth of *S. rolfsii* were increased as the quantity of tissue incorporated was increased (Table 4). Germination was stimulated equally by similar amounts of tissue from all cultivars. However, growth of *S. rolfsii* was not increased as much by high tissue rates of cultivar Florunner and Spanish selection C_2 compared to cultivars Florigiant, NC 2, and NC 3033. Partially decomposed peanut stems or leaves were not stimulatory to sclerotial germination or growth of *S. rolfsii* compared to the unamended treatments.

DISCUSSION

Although historical observation suggest *S. rolfsii* to be more severe in wet seasons (1), most peanut research workers recognize that epidemics frequently follow periods of drought. Smith (7) suggested that drying of sclerotia is the main trigger for germination in nature, resulting in the haphazard incidence of the disease in the field as moisture becomes available. Although sclerotia in this study were not dried to the extent of those used by Smith, germination of culture-grown sclerotia was increased by wetting in several tests as reported by Smith (Table 2). Wetting of soil-produced sclerotia, however, did not affect germination, indicating that Linderman and Gilbert (6) were correct in suggesting that sclerotia of *S. rolfsii* grown in culture may be physiologically different from those produced in field soil. Germination of soil-produced sclerotia was increased fivefold or more in the presence of remoistened peanut hay. Furthermore, wetting of sclerotia by soaking or leaching prior to exposure to peanut hay increased germination of field-produced sclerotia to a level similar to that observed with culture-grown sclerotia.

Previous studies by Linderman and Gilbert (5) indicated that growth of *S. rolfsii* was enhanced by volatile stimulants. A subsequent report (6) indicated that soil-produced sclerotia also responded to volatile stimulants that originated from alfalfa hay; ie, growth of *S. rolfsii* was increased. Soil-produced sclerotia in this study were stimulated by volatile compounds originating from dried peanut stems and leaves as well as finely ground hay. Each of the five peanut cultivars tested was highly effective in stimulating germination of sclerotia and growth of *S. rolfsii* with quantity of tissue comparable to that found beneath peanut plants growing in the field. However, no correlation was found between field performance and the same stimulatory capacity of these cultivars. In field tests, these same cultivars are known to range from highly susceptible to *S. rolfsii* (Spanish C_2) to moderately resistant (NC 3033).

It is obvious that water must be available for sclerotial germination, fungus growth, and infection; but rainfall or irrigation following dry weather does not consistently result in epidemics caused by *S. rolfsii*. Accumulation of dried leaves around the base of peanut plants partially defoliated by any stress factor (eg, leafspot disease, insect damage, or drought) creates optimal conditions for initiating an epidemic if sclerotia are present. Other environmental conditions (eg, continued moisture availability, conducive temperatures, presence of organic debris, etc.) influence subsequent development and severity of the epidemic.

LITERATURE CITED

1. AYCOCK, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. Agric. Exp. St. Tech. Bull. 174. 202 pp..
2. BACKMAN, P. A., and R. RODRIGUEZ-KABANA. 1976. Development of a medium for the selective isolation of *Sclerotium rolfsii*. Phytopathology 66:234-236.
3. CORNFIELD, A. H. 1961. A simple technique for determining mineralization of carbon during incubation of soils treated with organic materials. Plant Soil 14:90-93.
4. JACKSON, C. R., and D. K. BELL. 1969. Diseases of peanut (groundnut) caused by fungi. GA. Agric. Exp. St. Res. Bull. 56. 137 pp.
5. LINDERMAN, R. G., and R. G. GILBERT. 1969. Stimulation of *Sclerotium rolfsii* in soil by volatile components of alfalfa hay. Phytopathology 59:1366-1372.
6. LINDERMAN, R. G., and R. G. GILBERT. 1973. Behavior of sclerotia of *Sclerotium rolfsii* produced in soil or in culture regarding germination stimulation by volatiles, fungistasis, and sodium hypochlorite treatment. Phytopathology 63:500-504.
7. SMITH, A. M. 1972. Dry and wetting of sclerotia promotes biological control of *Sclerotium rolfsii* Sacc. Soil Biol. Biochem. 4:119-123.
8. SMITH, A. M. 1972. Nutrient leakage promotes biological control of dried sclerotia of *Sclerotium rolfsii* Sacc. Soil Biol. Biochem. 4:125-129.