Effect of Volatile Compounds From Remoistened Plant Tissues on Growth and Germination of Sclerotia of *Sclerotium rolfsii*

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ABSTRACT

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Germination of sclerotia and growth of *Sclerotium rolfsii,* which were stimulated by volatile compounds from remoistened, undecomposed plant tissues, were most pronounced when CO_2 was removed and O_2 added in 24-cm-diameter, 9.9 L desiccators. Germination of sclerotia and growth of *S. rolfsii* were increased by remoistening dried green tissue from alfalfa, corn, crotalaria, kudzu, lespedeza, peanut, and ramie. In a survey of 71 organic volatile compounds, two alcohols (including methanol), three aldehydes, two esters, and two halogenated hydrocarbons were as stimulatory as remoistened peanut hay to sclerotial germination of *S. rolfsii.* Methanol (about 2–4 μl/g hay) was detected by microdiffusion analysis of remoistened peanut hay. Germination of sclerotia of *S. rolfsii* was equal ($P = 0.05$) in the presence of methanol (7 μl) or peanut hay (2 g) in the desiccators. Addition of pectinase to 1% pectin also increased germination of sclerotia and growth of *S. rolfsii*; enzymatically released methanol may be a major stimulant emanating from remoistened, undecomposed plant tissue. The pectin-methanol hypothesis which is proposed in this study may explain, in part, the wide host range of *S. rolfsii.* These results also expand the list of known stimulants and inhibitors of sclerotial germination of *S. rolfsii.*

Additional key words: Arachis hypogaea, epidemiology, plant residue.

Volatile compounds from senescent peanut leaf litter may stimulate germination of sclerotia of *Sclerotium rolfsii* Sacc., and thus initiate epidemics of southern stem rot of peanut (*Arachis hypogaea* L.) (2). We were the first to report a correlation between volatile stimulants originating from intact peanut tissues and germination of field-produced sclerotia. A previous report, however, established that volatile compounds in a distillate of alfalfa hay may have stimulated germination and promoted growth of culture-grown sclerotia of *S. rolfsii* (6). Owens et al (8) suggested that aldehydes might be a major stimulant of fungal growth in soil by alfalfa hay (8).

Colonization of plant residue, which serves as a food base, was proposed to be a prerequisite for successful parasitism of host plants by *S. rolfsii* (3). Although most plant residues remaining from previous crops will serve as a substrate for growth of *S. rolfsii,* stimulation of germination of sclerotia has not been correlated with the presence of partially-decomposed organic debris. However, dried green leaves of five peanut cultivars, chosen to represent a range of susceptibility to *S. rolfsii,* were stimulatory to sclerotial germination (2).

The purposes of this study were: to determine the potential of undecomposed tissues from various plant species to stimulate germination of sclerotia of *S. rolfsii*, to characterize a spectrum of naturally available organic volatiles for their efficacy in stimulating sclerotial germination and fungal growth, and to determine the biological source of important volatiles that may be involved in sclerotial germination and, hence, in the initiation of southern stem rot epidemics.

MATERIALS AND METHODS

Assay for germination of sclerotia. Sclerotia were produced on *S. rolfsii*-infested oat grains in a sandy loam field soil as described previously (2). Sclerotia were placed on the surface of moistened soil plates in 24-cm-diameter desiccators (9.9 L) containing 5 g BaO_2 and 10 ml of water in a vial in the center of the supporting screen, and various plant tissue amendments in the bottom of the desiccators (2). After 48 hr the number of germinated sclerotia were counted and extent of mycelial growth measured according to a scale of 1–4, in which 4 represented maximal growth in control (2 g peanut hay per desiccator) and 1 represented no growth.

Evaluation of CO_2, O_2 and removal of organic compounds. Tests were conducted wherein the effects of removal of CO_2 or volatile organic compounds and enrichment of O_2 were compared with or without the presence of volatiles emitted from 2 g of moistened peanut hay placed in desiccator bottoms. Fifteen milliliters of 6N sodium hydroxide were placed in a petri dish positioned in the center of the desiccator to absorb CO_2. A solution of barium dioxide (5–10 g) plus 20 ml of water was added to desiccators in the same manner to simultaneously absorb CO_2 and release O_2 (5). The organic volatile trap consisted of 15 ml of potassium dichromate plus sulfuric acid (0.03 N $K_2Cr_2O_7$ plus 10 N H_2SO_4) in one dish and 15 cm^3 of activated carbon (Fisher Scientific, Pittsburgh, PA 15219) in a second dish (4).

Evaluation of plant residues. The following plant species were tested for stimulation of sclerotial germination in the desiccator system: *Arachis hypogaea*, *Bohemaria nivea* Jacq., *Crotalaria spectabilis* Roth, *Gossypium hirsutum* L., *Lespedeza stipulacea* Maxim., *Medicago sativa* L., *Pueraria thunbergiana* (Sieb. & Zucc.) Benth., and *Zea mays* L. Roots, stems, and leaves of *A. hypogaea* and cobs, hulls, and stalks of *Z. mays* were assayed.

In routine tests, 2 g of remoistened tissue were placed in a petri dish in the bottom of desiccators for each evaluation. In a test to determine the nature of the stimulant and effect of temperature on its release from peanut tissue, 4 g of finely-ground peanut hay was soaked in 40 ml of deionized water at 4 C for 16 hr. The leachate was tested for stimulation of germination of sclerotia in a dilution series of 100, 50, 25, 10, and 5% by adding 20 ml of leachate dilution to a petri dish in the bottom of desiccators. Unless specified otherwise, germination and growth data are reported as a percentage of the

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value for 2 g of dried green peanut hay, to standardize data between tests.

Evaluation of organic compounds. The following types of volatile compounds were tested for effect on germination of sclerotia and growth of *S. rolfsii*. *Alcohols:* methanol, ethanol, *n*-propanol, 2-propanol, allyl alcohol, *n*-butanol, iso-butanol, sec-butanol, tert-butanol, (2-methyl, 2-butanol), *n*-pentanol, 2-pentanol, 3-pentanol, iso-amyl alcohol, heptanol, octanol, dodecanol, octadecanol, and tetradecanol. *Aldehydes:* formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and heptaldehyde. *Esters*: methyl acetate, ethyl acetate, butyl acetate, propyl acetate, methyl propionate, propyl propionate, ethyl l-butyrate, allyl propionate, and allyl-*n*-butyrate. *Ketones*: acetone, pentanone, butanone, dimethyl butanone, and heptanone. *Hydrocarbons*: pentane, hexane, heptane, octane, and kerosene. *Halogenated hydrocarbons*: dichloromethane, chloroform, carbon tetrachloride, dichloroethane, trichloroethane, tetrachloroethane, l-chloropropane, 2-chloropropane, 1, 2-dichloropropane, 1-bromopropane, 2-brompropane, dibromochloropropane, 1-bromohexane, ethyl iodide, 1-iodopropane, and 2-iodopropane. *Acids*: formic acid, acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, heptanoic acid, nonanoic acid, and caprylic acid. *Miscellaneous*: furfural and carbon disulfide.

One 6-mm-diameter Whatman No. 2 filter disk dipped in the test compound (about 2.9 mg chemical) was added to the bottom of 24-cm-diameter desiccators. Moistened peanut hay (2 g) and deionized water were included in each test as a reference.

Source of stimulant in peanut hay. The stimulatory effect of peanut hay and methanol on germination of sclerotia and growth of *S. rolfsii* were compared in dilution series. Peanut hay at 1,000, 500, 250, 120, 60, and 30 mg/desiccator was compared with methanol at 500, 250, 120, 60, 30, 7 and 3.5 μl/desiccator. Because methanol is released by enzymatic degradation of pectin (7), a test was conducted using 200 g of reagent grade sea sand, sandy loam field soil, or water in the bottom of desiccators. Ten milliliters of 1% pectin solution with or without 10 ml of pectinase (2 mg/ml) (Nutritional Biochemicals Corp., Cleveland, OH 44128) was added to sand, soil, or in petri dishes in the bottom of the desiccators. Two grams of dried green peanut hay, 0.1 ml of methanol, water, moist sand, or soil in similar petri dishes were included as controls. All other procedures were as previously described. Germination of sclerotia and growth of *S. rolfsii* were determined after 48 hr.

Chemical analysis. The amount of methanol released from peanut tissues was determined by Conway microdiffusion into 10% (v/v) H_2SO_4 followed by oxidation with permanganate to formaldehyde and reaction with chromotropic acid (4). Determination of acetaldehyde was by the semicarbazide procedure of Burbridge, Hine and Schick (4). Pectin methyl esterase (PME) activity in soil was determined by incubating 10 g of Norfolk sandy loam with 5 ml of 1% (w/v) citrus pectin in a 60-ml bottle for 16 hr at 37 C. After incubation, 50 ml of water was added and methanol in 2 ml of the suspension was determined by Conway microdiffusion into 10% H_2SO_4 followed by reaction with chromotropic acid. The driving solution for microdiffusion was a 1 M phosphate buffer (pH 6.2) in saturated sodium chloride. Diffusion was performed at room temperature for 16 hr. Controls with autoclaved soil or water instead of pectin were included. Determinations were performed in triplicate at $A_{580\ nm}$ with a Model 139 Perkin-Elmer spectrophotometer.

Effect of temperature on germination of sclerotia in soil amended with peanut tissues. Ten sclerotia were placed in the bottom of 100-ml beakers and moistened field soil (Norfolk sandy loam) was added to a height of 2 cm after compaction. Treatments consisted of 1% finely ground peanut hay mixed in soil (w/w) and 0.5 g dried green peanut stems placed on the surface of soil. Control beakers received soil without amendments. Three beakers for each treatment were incubated at 22, 24, 26, 28, or 30 C. Each beaker was incubated individually in a small plastic bag. The number of germinated sclerotia was determined by observation through the glass bottom and sides of the beakers. Growth of *S. rolfsii* to the surface of soil and production of new sclerotia were determined after 6 days. A second test was conducted at 22, 24, 26, 28, and 30 C in 5-cm-diameter petri dishes in which sclerotia were placed in moistened soil l, 2, or 3 cm from a 2-cm-long dried green peanut stem (about 60 mg). Control plates received no amendment. Germination of sclerotia was determined after 48 hr.

Use of stimulants to increase infection of tomato by *S. rolfsii*. Two tomato (*Lycopersicum esculentum* Mill. 'Rutgers') plants were transplanted, 2 wk after emergence, into 11-cm-diameter plastic pots containing 500 g of field soil (Norfolk sandy loam) and grown under greenhouse conditions. Ten sclerotia were placed on the soil surface and the following amendments were added: 500 mg of dried green peanut hay, 375 mg blackstrap molasses (2.5% solution), 50 mg citrus pectin, or 100 μl methanol (1.0% solution). Control pots received deionized water. Six pots receiving each type of amendment were treated as follows: immediate application of the fungicide carboxin (5,6-dihydro-2-methyl-l,4-oxathiin-3-carboxanilide) at a rate equivalent to 1.12 kg/ha (atomized on soil surface in 1 ml of water); application of carboxin 3 days after amendments; or no fungicide. Each pot was covered with a plastic bag to maintain high humidity. Six days after inoculation, percentage of germinated sclerotia, percentage of sclerotia visibly colonized by *Trichoderma* sp. and other fungal parasites, and the number of infected plants were recorded.

RESULTS

Effect of removal of CO_2 and organic volatiles and addition of O_2 on germination of sclerotia and growth of *S. rolfsii* in the presence of remoistened peanut hay. Germination of sclerotia was increased ($P = 0.05$) by the presence of NaOH (removal of CO_2 from atmosphere) and BaO_2 (removal of CO_2 and simultaneous release of O_2) in desiccators containing moistened peanut hay (Table 1). Only in the presence of BaO_2, however, was the growth of *S. rolfsii* increased above that observed in desiccators containing moistened peanut hay. The organic volatile trap negated the enhancing effect of peanut hay on germination; ie, germination was equal to that in the control, in the presence of BaO_2 and the organic volatile trap without peanut hay. Presence of NaOH without peanut hay was somewhat toxic as evidenced by lower germination and fungal growth. BaO_2 and the organic volatile trap did not affect percent germination or growth of *S. rolfsii* when no amendments were included.

Effect of plant residues. Sclerotial germination was greatest in the presence of *B. nivea* (ramie) hay, lespedeza hay, and dried green peanut hay, intact stems or leaves. The previously mentioned tissues plus *P. thunbergiana* (kudzu) hay gave maximal growth of *S. rolfsii*. Dried peanut roots, hulls, and fresh green peanut stems and leaves were not stimulatory to sclerotial germination or growth of *S. rolfsii* as compared to the unamended treatments. However,

TABLE 1. Germination of sclerotia of *Sclerotium rolfsii* as affected by removal of CO_2, organic volatiles, and the addition of O_2

Treatment[x]	Percent germination[y]		Growth index[z]	
	without hay	with hay	without hay	with hay
6N NaOH	13 d	87 a	2.1 h	2.8 f
$K_2Cr_2O_7$-H_2SO_4 + activated carbon	34 c	37 c	2.3 g	2.8 f
BaO_2	29 c	91 a	2.3 g	3.8 e
Control	37 c	53 b	2.3 g	3.0 f

[x] Each 24-cm-diameter desiccator received one of the following in petri dish: 15 ml 6N NaOH (removal of CO_2), 15 ml of $K_2Cr_2O_7$-H_2SO_4 plus 15 cm^3 activated carbon in a separate dish (removal of organic volatiles), 10 g BaO_2 in 20 ml water (simultaneous removal of CO_2 and addition of O_2), or 10 ml of deionized water.

[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 an average rating for all germinated sclerotia after 48 hr according to a scale of 1 to 4, in which 4 represents maximal growth of control (2g peanut hay per desiccator) and 2 represents germination but little or no mycelial extension.

germination of sclerotia and growth of *S. rolfsii* were intermediate with *C. spectobilis* hay, *M. sativa* (alfalfa) hay, and the shucks, stalks, and cobs of *Z. mays* (corn).

No reduction in sclerotial germination or growth of *S. rolfsii* was found when BaO_2 was used in the desiccator containing soil plates exposed to peanut hay for 7 days prior to implanting sclerotia and exchanging the original peanut hay with an equal amount of moistened hay (Table 2).

Effect of organic compounds. The stimulant effects of two alcohols (methanol and octanol) three aldehydes (acetaldehyde, propionaldehyde, and heptaldehyde), two esters (butyl acetate and ethyl 1-butyrate), and two halogenated hydrocarbons (tetrachloroethane and dibromochloropropane) were equal to that of peanut hay ($P = 0.05$) on both germination of sclerotia and growth of *S. rolfsii* in soil plates. Eight compounds were inhibitory to germination of sclerotia and/or growth of *S. rolfsii* (allyl alcohol, tert-butanol, 2-methyl 2-butanol, *n*-pentanol, 2-pentanol, formaldehyde, allyl propionate and allyl-*n*-butyrate). Twenty compounds had no effect on sclerotial germination or growth of *S. rolfsii* at concentrations tested (*n*-butanol, sec-butanol, tetradecanol, butanone, dimethyl butanone, pentane, octane, kerosene, dichloromethane, 2-chloropropane, 1,2-dichloropropane, 1-bromopropane, ethyliodide, 1-iodopropane, 2-iodopropane, heptanoic acid, nonanoic acid, caprylic acid, furfural, and carbon disulfide). The remaining 34 compounds were stimulatory either to germination of sclerotia or to fungal growth, but not both.

Source of volatile stimulant in peanut hay. Methanol at 7–500 μl/desiccator was equal to peanut hay at 120–1,000 mg in capacity to stimulate germination. All dilutions of methanol and peanut hay enhanced germination as compared to unamended control. Stimulation of growth of *S. rolfsii* with peanut hay at 500–1,000 mg was equal to methanol at 30–250 μl/desiccator. Growth of *S. rolfsii* in the presence of methanol at 3.5–7 μl was equal to peanut hay at 30–120 mg/desiccator. Leachates of dried peanut hay collected after soaking in water for 16 hr at 4 C did not stimulate germination of sclerotia or growth of *S. rolfsii* when added to the bottom of desiccators at any dilution.

Enhancement of sclerotial germination by both methanol and peanut hay was equivalent ($P = 0.05$) to the combined effect of pectin plus pectinase in moist soil, in sand, or in petri dishes (Table 3). Pectinase alone in either moist sand or petri dishes stimulated germination to a lesser degree. Growth of *S. rolfsii* was increased most in the presence of methanol, peanut hay and pectin plus pectinase in petri dishes. Lesser stimulation of growth was noted for pectin plus pectinase in moist sand and soil. Other treatments alone or in combination did not differ from the control.

Chemical analysis. No acetaldehyde was detected in microdiffusion tests in which remoistened, dried peanut hay was used. Tests were positive, however, for methanol. Methanol (about 2–4 μl/g hay) was trapped in H_2SO_4 in the presence of remoistened, dried peanut hay but no methanol was detected in the presence of moistened, partially decomposed peanut hay. Autoclaved dried peanut hay emitted less, but detectable, amounts of methanol. Autoclaved partially decomposed peanut hay emitted even lower quantities of methanol.

Effect of temperature on germination of sclerotia in soil amended with peanut tissues. Sclerotia germinated at 22–30 C after 6 days in soil supplemented with 1% peanut hay or 0.5 g dried peanut stems on the soil surface. Sclerotia did not germinate at any temperature in the unamended controls during the test. At 26–30 C, *S. rolfsii* grew to the soil surface in 6 days in the treatment with 1% peanut hay. New sclerotia were produced profusely throughout the soil in this treatment. Only at 30 C did *S. rolfsii* grow to the new soil surface after 6 days in the treatments that received peanut stems. New sclerotia were not observed in these treatments.

Percentage germination of sclerotia increased as temperature increased in soil plates that received 2-cm-long dried peanut stems.

TABLE 2. Effect of exposing soil plates to volatile compounds emanating from peanut hay for 7 days prior to placing sclerotia and determining germination of sclerotia and growth of *Sclerotium rolfsii* (in the presence or absence of additional peanut hay)

Treatment[x]			
0 day	7 day	Germination (%)	Growth index[y]
Peanut hay	Peanut hay	100 a	3.7 c
Peanut hay	(None)	28 b	2.0 d
(None)	Peanut hay	92 a	3.6 c
(None)	(None)	34 b	2.1 d

[x] Two grams of moistened peanut hay was placed in bottom of 24-cm-diameter desiccators containing seven soil plates and BaO_2 (10 g plus 20 ml water) in a separate dish. Original hay (0 day) was removed after 7 days of incubation, prior to exchanging with additional peanut hay and implanting 10 sclerotia per plate.

[y] Index is average rating for all germinated sclerotia after 48 hr according to a scale of 1 to 4, in which 4 represents maximal growth of control (2 g peanut hay per desiccator) and 2 represents germination but little or no mycelial extension. Numbers followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Effect of enzymatic (pectinase) degradation of pectin on stimulation of sclerotial germination and growth of *Sclerotium rolfsii*

Treatment[w]	Substrate[x]	Germination[y] (%)	Growth index[z]
Peanut hay	···	67 a	3.5 g
0.1 ml Methanol	···	70 a	3.2 g
Pectin	Sand	29 de	2.0 j
Pectin	Soil	31 de	2.1 ij
Enzyme	Sand	43 bc	2.0 j
Enzyme	Soil	29 de	2.0 j
Pectin + enzyme	Sand	60 a	2.4 h
Pectin + enzyme	Soil	57 ab	2.4 h
Pectin	···	31 de	2.1 ij
Enzyme	···	34 cd	2.0 j
Pectin + enzyme	···	53 ab	3.2 g
	Sand	27 de	2.0 j
	Soil	13 f	2.0 j
Water	···	17 e	2.0 j

[w] Pectin added as 10 ml of 1% solution and pectinase as a 10% solution (2 mg/ml) directly to sand, soil, or in petri dish in the bottom of 24-cm-diameter desiccators.

[x] Sea sand and field soil (200 g) added to bottom of desiccators.

[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 rating for all germinated sclerotia after 48 hr according to a scale of 1 to 4, in which 4 represents maximal growth of control (2 g peanut hay per desiccator) and 2 represents germination, but little or no mycelial extension.

TABLE 4. Effect of several soil amendments with and without a fungicide treatment on germination of sclerotia of *Sclerotium rolfsii*

	No fungicide		Fungicide treated	
Treatment[x]	Sclerotia germinated[y] (%)	Tomatoes killed (%)	Parasitized sclerotia[z] (%)	Tomatoes killed (%)
Peanut hay	88 a	100 c	86 e	0
Molasses	79 a	92 c	63 e	0
Pectin	63 a	100 c	84 e	0
Methanol	72 a	100 c	86 e	0
Control	28 b	33 d	21 f	0

[x] Dried peanut hay added at 500 mg/pot, molasses added at 375 mg (2.5% solution) per pot, citrus pectin added at 50 mg/pot and methanol added at 100 μl (1.0% solution) per pot.

[y] Determinations made in control pots 6 days after inoculation. Numbers followed by a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

[z] Determinations made 6 days after inoculation in pots which were treated with carboxin at time of inoculation. *Trichoderma* sp. were observed on all parasitized sclerotia.

After 48 hr germination was 2, 4, 22, 24, and 51%, respectively, at 22, 24, 26, 28, and 30 C. At 30 C, germination of sclerotia and further growth of *S. rolfsii* at 1 cm was greater than those of sclerotia placed at 3 cm from implanted stems. At lower temperatures, however, stimulation of germination was not associated with closeness to buried stems. Sclerotia did not germinate at any temperature in unamended soil plates during the test.

Effect of stimulants on infection of tomato by *S. rolfsii*. Application of dried peanut hay, molasses, citrus pectin, and methanol to soil in pots containing tomato all resulted in approximately three-fold increase in sclerotial germination ($P = 0.05$) over the unamended controls (Table 4). Similarly, a three-fold increase in infected plants ($P = 0.05$) occurred when all four amendments were added to soil in pots infested with 10 sclerotia. Application of carboxin to all amended treatments resulted in a four-fold increase in the number of sclerotia that were colonized by other fungi (mainly *Trichoderma* sp.). Tomato plants remained healthy in all carboxin treatments.

DISCUSSION

Stimulation of sclerotial germination and growth of *S. rolfsii* by volatile compounds emitted from dried undecomposed tissues of many plant species after remoistening is probably a common phenomenon in nature. Data presented in these studies suggest that methanol, originating from remoistened peanut leaves, may be a major stimulus for germination of sclerotia, functioning as a trigger in initiating epidemics of southern stem rot in peanut fields. Excised green leaves or vigorously growing peanut stems did not produce volatile stimulants for sclerotial germination or growth of *S. rolfsii*. These conclusions relate positively to field observations that *S. rolfsii* is not stimulated to germinate during periods of vigorous growth by *A. hypogaea*, but epidemics of southern stem rot often occur as peanut plants begin to mature or after severe environmental stress (eg, drought) when defoliation occurs.

Methanol was very effective in stimulating sclerotial germination and *S. rolfsii* growth. The large amounts of methanol emitted from dried peanut hay (about 2–4 μl/g hay) corresponds with analysis of volatile components in alfalfa distillate (8). In contrast with alfalfa distillate, however, no acetaldehyde was detected from peanut hay. The possibility of ethylene as a stimulant was discounted when no effect on germination was noted at 12.5, 25, 50, 100, 150, 200, and 400 μg/ml 48 hr after a 0.3 ml solution of ethephon (21.3% 2-chloroethyl phosphonic acid) was sprayed on soil plates containing 10 sclerotia (M. Beute, *unpublished*).

Pectin methyl esterase occurs universally in plant tissues, primarily absorbed on the cell walls. PME is also very resistant to drying and heat inactivation in its natural (cell-wall-absorbed) state in plant tissues (7). Pectin and pectinlike compounds are common constituents of undecomposed plant walls. Low molecular weight aldehydes and alcohols also are common constituents of many plant tissues (1). Our data suggest that senescence or death of green peanut tissues triggers enzymatic (or autocatalytic) reactions which result in the release of methanol and other volatile stimulants. This suggestion is supported by the comparative efficacy of dried peanut hay, methanol and the pectin plus pectinase combination in stimulating sclerotial germination and growth of *S. rolfsii*. Because of the ubiquitous distribution of pectin and PME in plant tissues, the pectin-methanol system helps to explain the stimulation of sclerotial germination by undecomposed tissues of different sources. The pectin-methanol system may also explain, in part, the wide host range of the pathogen.

The stimulatory effect of methanol when added to soil containing 10 sclerotia in the greenhouse tomato test was equal to peanut hay, pectin and molasses. Molasses was a very effective stimulant for sclerotial germination and provided energy for growth of *S. rolfsii* but did not emit volatile stimulants in desiccator tests. Peanut hay in the tomato test may have stimulated sclerotia by direct nutrient diffusion or by volatiles. Pectin also may have functioned as a food source for *S. rolfsii* but, in addition, may be a source of methanol as microbial degradation proceeds. In these studies disease incidence was equal with all four types of amendments, suggesting that methanol both stimulated sclerotia germination and provided a source of energy for infection. Increased parasitism of sclerotia by *Trichoderma* sp. in treatments receiving both stimulatory amendments and carboxin indicates a potential for the use of stimulatory compounds in reducing *S. rolfsii* inoculum in infested soil. Heavily colonized sclerotia, resulting from carboxin treatments, did not decay if dried prior to storage in the laboratory but all were rotted in 2–3 wk if left in moist soil. Even though *S. rolfsii* may be stimulated to germinate in a field situation, infection of older, more resistant peanut stems may necessitate the presence of a nutrient substrate as suggested by Boyle (3).

Our studies substantiate a previous report (6) that *S. rolfsii* sclerotia can be stimulated at a soil depth of 2-cm or more, germinate and grow to a nutrient substrate. The effect of stimulation and subsequent fungal growth was influenced by temperature and moisture. Even if sufficient moisture is available, the probability of a specific sclerotium germinating becomes less as temperature decreases from 30 C. If colonization of peanut tissue has occurred and new sclerotia formed, subsequent stimulation from newly defoliated leaves will trigger additional sclerotial germination, initiating an epidemic of southern stem rot if environmental conditions are conducive.

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