Effect of Volatile Compounds, Nutrients, and Source of Sclerotia on Eruptive Sclerotial Germination of *Sclerotium rolfsii*

Z. K. Punja, S. F. Jenkins, and R. G. Grogan

Research scientist, Campbell Soup Company (also visiting assistant professor) and professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695; and professor, Department of Plant Pathology, University of California, Davis 95616, respectively.

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ABSTRACT

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Sclerotia of *Sclerotium rolfsii* produced on oat kernels in the laboratory were stimulated to germinate eruptively by alcohols and acetaldehyde at levels ranging from 1.6 to 6.1 μl per petri dish. Isopropyl and butyl alcohols were more stimulatory to germination than volatiles evolved from dried and remoistened alfalfa leaf tissue. These compounds increased linear growth rate of the mycelium but did not significantly affect dry weight. The addition of a carbon source, such as 20 mM glucose or sucrose, to Noble water agar inhibited eruptive germination but stimulated hyphal germination. Dry weight loss from sclerotia and leakage of amino compounds and carbohydrates during germination were reduced by glucose. Sclerotia from potato-dextrose agar cultures responded differently to selected alcohols and NaClO treatment than sclerotia produced on oats in the laboratory or in soil. Differences among these sclerotia in the thickness and composition of the rind and underlying cortical tissue were observed which could in part have accounted for these behavioral differences. Low and inconsistent germination of sclerotia produced in soil was attributed to the presence of contaminating microorganisms on the sclerotial surface.

Sclerotia of *Sclerotium rolfsii* were reported to germinate eruptively following drying (20), exposure to volatile compounds (primarily alcohols and aldehydes) evolved from plant tissues (5,6,15,16,20) and to a lesser extent following brief NaClO treatment (16,20). Among numerous alcohols tested in vitro, only methanol and octanol were reported to significantly increase germination of sclerotia (5). Owens et al (18), however, observed only slight increases in growth of soil fungi in the presence of methanol or ethanol; the greatest increases were obtained with alcohols containing three to six carbon atoms (18). Many other volatile compounds also may be released from plant tissues (3,7,11) and from germinating seeds (13,14,25,27). A number of these have been reported to stimulate germination of propagules and increase growth of fungi (11–14, 18, 25) and some induced chemotactic (2) and chemotropic (15,17,21) responses. The type of response (stimulation or inhibition) of fungi to these compounds depends on the concentration at which they are used; while many are stimulatory at low (nanograms per milliliter) to moderate (micrograms per milliliter) concentrations, almost all are inhibitory at high concentrations (11–13,15,18,23).

Assays of sclerotial germinability of *S. rolfsii* in previous studies have been conducted on field soil (5,6,16,20), agar (20), and on substrates free of nutrients such as glass filters and quartz sand (6,20). Incubation of sclerotia on media containing nutrients was reported to inhibit eruptive germination (20), but the mechanism of inhibition was not determined. Thus, the influence that exogenous nutrients in assay substrates may have on eruptive germination is not known.

In many studies on sclerotial germination of *S. rolfsii*, sclerotia from laboratory cultures were employed as they germinated consistently and were free of microbial contamination (15,20,21). Some investigators proposed, however, that these sclerotia differed from those formed in unsterile soil (6,16). Differential sensitivity to prolonged NaClO treatment suggested that structural and/or physiological differences could have been present (16). However, the general responses of both types of sclerotia (culture and soil) to volatile compounds (15,16,20) and leaching treatments (6) were similar, although sclerotia produced in soil generally exhibited much lower and less consistent germination (5,6) than those from sterile culture. The bases for these apparent differences have not been resolved.

Since sclerotia of *S. rolfsii* are the principal means of survival of the fungus in soil and are capable of initiating infection upon germination (21), information on factors affecting their germination is essential to understanding or interpreting the infection behavior of this pathogen. The objectives of this study were to determine the effects of, and possible mechanisms by which, selected volatile compounds and exogenous nutrients influenced eruptive germination of sclerotia of *S. rolfsii*. Sclerotia produced in the laboratory and in soil were compared for germinability responses to alcohols and NaClO treatment on two assay substrates, and for possible morphological or structural differences. The extent of dry weight loss from sclerotia in the presence and absence of nutrients and volatile compounds also was determined.

MATERIALS AND METHODS

Assessment of sclerotial germination. The sources of the four isolates (159, 1003, 1120, and 2672) of *S. rolfsii* that were used and the method of producing sclerotia on autoclaved oat kernels in the laboratory were described previously (22). Sclerotia from 2- to 6-mo-old cultures were either air-dried for 10–20 hr at 15–20% relative humidity or over $CaCl_2$ in a desiccator prior to use to induce eruptive germination (20), or were used without drying. Sclerotial

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germination in most experiments was assayed on 1% Noble water agar (Difco Laboratories, Detroit, MI 48232). About 10 ml was poured into each 60 × 15-mm petri dish, inoculated with 25 sclerotia, and incubated at 28 C in the dark for 3–5 days. In some experiments, germination also was assayed on the surface of unsterilized G-1 field soil; the characteristics of this soil were described previously (22). Eruptive germination was distinguished from hyphal germination (20) by squeezing germinated sclerotia with a pair of forceps after 5–7 days of incubation; collapse of the sclerotial rind, which is evidence for utilization of internal stored materials (20), indicated eruptive germination. The data presented are the means of four replicates and all experiments were repeated twice. Differences among treatment means were analyzed at a significance level of $P = 0.05$ according to Fisher's LSD test.

Effect of volatile compounds on germination of laboratory-produced sclerotia. Fifteen compounds (Table 1), each at 0.25 M concentration, were tested for ability to stimulate eruptive germination on Noble agar of nondried sclerotia from oat cultures. At this molarity, the solutions ranged in concentration from 0.8% (v/v) (for methyl alcohol) to 3.05% (for phenethyl alcohol). Two-tenths of 1 ml was added to a 1-cm-wide × 0.5-cm-high glass vial which was placed in a well in the center of the petri dish (volume = 30 cm^3). The actual amount of each compound added to the dish was calculated from: mol. wt. × 0.25/1,000 × 0.2 ml; the levels ranged from 1.6 to 6.1 μl (Table 1). Sclerotia were placed radially 1.0 and 2.0 cm from the vial. Control dishes received sterile distilled water. Alfalfa hay, autoclaved hay (30 min at 121 C), or hay with 15% (w/w) activated charcoal (Darco® G-60, Matheson Coleman and Bell, Norwood, OH 45212) also were included. Dishes containing similarly treated sclerotia were placed in polyethylene bags which were tightly sealed.

Effect of volatile compounds on mycelial growth. Solidified Noble agar in 100 × 15-mm petri dishes was overlaid with 90-mm-diameter cellophane pieces of uniform weight. Six dried sclerotia or a 6-mm-diameter mycelial plug taken from the margin of a 7-day-old potato-dextrose agar (PDA) culture was placed at the periphery of the dish. A vial containing 0.2 ml of 0.25 M isopropyl or butyl alcohol, or acetaldehyde, was placed about 80 mm away. Measurements on the extent of linear growth across the dish were made after 4 days. The cellophane was then removed, dried for 18 hr at 90 C, and weighed to obtain dry weight. The data are expressed as a percentage of the growth in control dishes that had received sterile distilled water.

Effect of nutrients. Four carbon sources, three amino acids, and combinations of glucose with DL-asparagine, sodium acetate, or adenosine 3',5'-cyclic monophosphate (cyclic AMP) (Sigma Chemical Co., St. Louis, MO 63178) in sterile distilled water were mixed with autoclaved 2% Noble agar; concentrations ranging from 10 to 50 mM of each of these compounds were tested. Sclerotial exudates were obtained from 200 dried sclerotia that were incubated for 18 hr in 25 ml of distilled water. The exudate was sterilized by passage through a 0.45 μm Millipore filter prior to incorporation into cooled agar. Germination of dried sclerotia on these substrates was compared to germination on unamended Noble agar.

Dry weight loss and leakage from sclerotia. Batches (1-g fresh weight) of nondried or dried (for 12 hr over $CaCl_2$ in a desiccator) sclerotia were placed on filter paper disks (40-mm-diameter) in 100 × 15-mm petri dishes. The disks were moistened either with a 50-mM glucose solution or with water. A vial containing alfalfa hay was placed in one-half of the dishes that had received water. Two replicate samples of sclerotia from each treatment were assayed every 12 hr for 72 hr. One sample was washed for 5 hr in water, dried at 95 C, and weighed to determine if changes in dry weight had occurred following exposure to the treatments. The other sample was assayed for the extent of leakage of soluble carbohydrates and amino compounds by using the methods described in an earlier study (20).

Comparison of soil and laboratory-produced sclerotia. *Germination.* Sclerotia produced in soil in the greenhouse according to the method of Beute and Rodriguez-Kabana (6) and from naturally infected carrots in the field were compared with laboratory-produced sclerotia for germination on Noble agar and field soil in the presence of aqueous solutions (v/v) of 1.2% methyl alcohol (methanol), 1.2% butyl alcohol (butanol), and after 0.5% NaClO treatment for 3 min. Sclerotia from 2-mo-old PDA and oat cultures were either air-dried for 24 hr at about 40% relative humidity or used without drying. Sclerotia from soil were air-dried.

Morphology and structure. Nondried sclerotia from laboratory cultures and from soil were fixed overnight in formalin-propionic acid-isopropyl alcohol, dehydrated through a graded isopropyl alcohol series, and infiltrated and embedded in Paraplast® (Sherwood Medical, St. Louis, MO 63103). Sections (10–12 μm) were made with a rotary microtome, stained with modified Conant's consisting of crystal violet-fast green-orange G and examined at ×100 magnification.

RESULTS

Effect of volatile compounds on germination of laboratory-produced sclerotia. Volatiles from alfalfa hay significantly ($P < 0.05$) increased eruptive germination on Noble water agar of nondried sclerotia (Table 1). The stimulatory effect was not reduced by autoclaving the hay, but it was annulled by adding activated charcoal. The eight alcohols tested all increased germination over the control; the least stimulatory among these was methyl alcohol. Exposure to isopropyl or butyl alcohol consistently gave 100% germination. Among the aldehydes, only acetaldehyde was stimulatory. At higher concentrations, both acetaldehyde and methyl alcohol reduced germination (Table 1). Although actual percent germination values of other isolates were different, the general responses of these isolates to the volatile compounds were similar.

Effect of volatile compounds on mycelial growth. Linear growth in the presence of isopropanol, butanol, or acetaldehyde was 120–130% of that in the water controls. The response of sclerotia and mycelial plugs was similar. There was no measurable increase, however, in mycelial dry weight over the controls in the presence of these volatile compounds under the conditions of this study.

TABLE 1. Eruptive germination of nondried sclerotia of *Sclerotium rolfsii* on Noble water agar in the presence of volatile compounds

Source of volatiles[w]	Amount[x]	Germination (%)[y]
Alfalfa hay	50 mg	92 b[z]
Autoclaved hay	50 mg	87 b
Hay + activated charcoal (15%, w/w)	50 + 7.5 mg	31 g
Activated charcoal	7.5 mg	27 g
Methyl alcohol	1.6 μl	48 f
	6.4 μl	10 i
Ethyl alcohol	2.2 μl	71 d
Isopropyl alcohol	3.0 μl	100 a
Butyl alcohol	3.6 μl	100 a
Pentyl alcohol	4.4 μl	61 e
Isopentyl alcohol	4.4 μl	59 e
Hexyl alcohol	5.1 μl	79 c
Phenethyl alcohol	6.1 μl	81 bc
Formaldehyde	1.5 μl	0 j
Acetaldehyde	2.2 μl	73 d
	4.4 μl	8 i
Glutaraldehyde	2.5 μl	16 h
Acetone	2.9 μl	26 g
Acetic acid	3.0 μl	30 g
Dichloromethane	4.2 μl	62 e
None		26 g
LSD ($P = 0.05$)		7.6

[w] All compounds were tested at a 0.25 M concentration.

[x] 0.2 ml was added to a 1-cm × 0.5-cm glass vial which was placed in a well cut in the agar in the center of a 60 × 15-mm petri dish.

[y] Germination was rated after three days of incubation at 28 C in the dark. Data are the means of four replicates of 25 sclerotia each; the experiment was repeated twice.

[z] Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's LSD test.

Effect of nutrients. Eruptive germination was inhibited by 10–50 mM glucose, fructose, or sucrose (Table 2) and hyphal germination was observed instead. Mannitol, amino acids, and sclerotial exudates did not affect eruptive germination. Addition of 20mM asparagine or Na acetate with glucose did not reverse the inhibitory effect. In the presence of 4 mM cylic AMP there also was no significant ($P \leq 0.05$) increase in the percentage of eruptive germination.

Dry weight loss and leakage from sclerotia. Dry weight loss from nondried control sclerotia was gradual and reached 12% after 72 hr of incubation (Fig. 1). Addition of glucose increased mycelial growth, which resulted in an increase in total dry weight. Dry weight loss from nondried sclerotia in the presence of alfalfa hay was greater than that in the control only after 36 hr; this increase was associated with increased exudation of amino compounds and carbohydrates. Drying sclerotia enhanced leakage of these water-soluble compounds and an 18% dry weight loss over the nondried control was recorded (following washing) prior to the onset of eruptive germination (Fig. 1). During a 72-hr incubation period, dry weight loss was increased by an additional 17%. The extent of weight loss was reduced in the presence of alfalfa hay or glucose. Total dry weight with glucose after 72 hr was comparable to that at the onset of germination and there was no detectable leakage.

TABLE 2. Eruptive and hyphal germination of dried sclerotia of *Sclerotium rolfsii* on Noble water agar amended with various nutrients

Nutrient[w]	Germination (%)[x]	
	Eruptive	Hyphal
Glucose	10 b[z]	90 a[z]
Glucose + asparagine[y]	8 b	92 a
Glucose + Na acetate[y]	6 b	94 a
Glucose + 4×10^{-3} M cyclic AMP	14 b	86 a
Fructose	8 b	92 a
Sucrose	5 b	95 a
Mannitol	95 a	4 b
Asparagine	96 a	4 b
Histidine	97 a	3 b
Leucine	91 a	8 b
Sclerotial exudate	92 a	8 b
None	97 a	3 b

[w] Nutrients were added to sterile distilled water and then mixed with autoclaved 2% Noble water agar prior to pouring into 60 × 15-mm petri dishes. The data presented are for the 20 mM concentration.

[x] Germination was rated after 5 days of incubation at 28 C. Data are the means of four replicates of 25 sclerotia each and the experiment was repeated twice.

[y] Asparagine and Na acetate were tested at 20 mM.

[z] Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's LSD test.

Comparison of soil and laboratory-produced sclerotia. On Noble agar and field soil, germination of dried sclerotia produced in the laboratory on PDA or oats was consistently higher than that of sclerotia from soil; overall germination was higher on agar than on soil (Table 3). Germination of nondried laboratory-produced sclerotia was stimulated more by drying than by exposure to methanol, butanol, or NaClO; the least stimulatory treatment was methanol. Sclerotia from oats germinated better than those from PDA. On field soil, germination of dried sclerotia produced on oats was significantly ($P = 0.05$) increased by butanol and NaClO, but not by methanol. Dried sclerotia from PDA also were stimulated by butanol, but NaClO treatment reduced germination (Table 3). On agar, germination of dried sclerotia from oats was 100% irrespective of the treatment. In the presence of butanol, there was abundant proliferation of aerial mycelium and no secondary sclerotia formed (Fig. 2). In comparison, germination on agar of dried sclerotia from PDA was reduced by these treatments (Table 2).

Germination of sclerotia from soil was stimulated more by NaClO treatment than by methanol or butanol. Overall germination was higher for sclerotia produced in soil in the greenhouse (6) than for sclerotia obtained from natural field soil (Table 3). Germination on soil of sclerotia produced in the greenhouse in the presence of water, methanol, or butanol was 29, 75, and 95%, respectively, of that of sclerotia treated with NaClO. The comparable values for field sclerotia were 27, 53, and 98%, respectively. Germination on agar was slightly higher (Table 3).

Morphology and structure. Three distinct layers (rind, cortex,

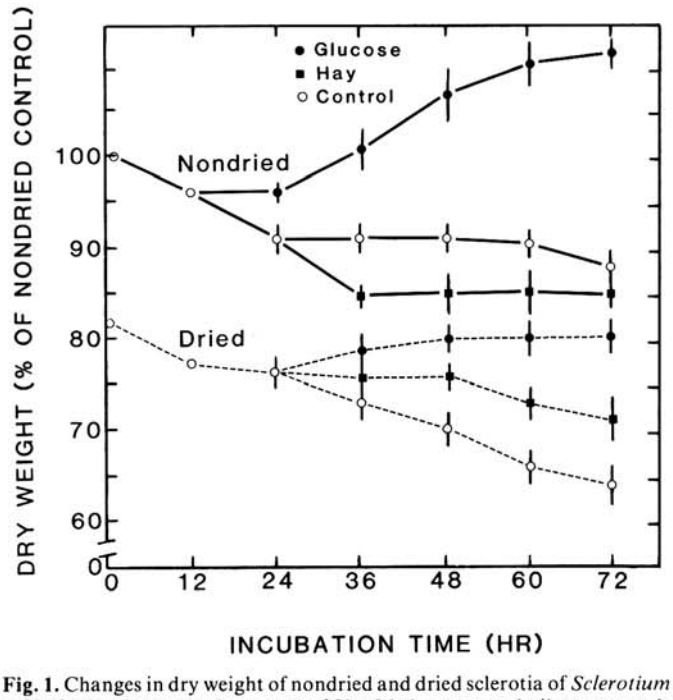

Fig. 1. Changes in dry weight of nondried and dried sclerotia of *Sclerotium rolfsii* in the absence and presence of 50 mM glucose or volatile compounds from alfalfa hay. Replicate samples of sclerotia were retrieved at 12-hr intervals and washed in water for 5 hr and dried, and then weighed. The dry weights are expressed as a percentage of the dry weight of the untreated control. Vertical bars represent standard deviations of the mean ($n = 6$).

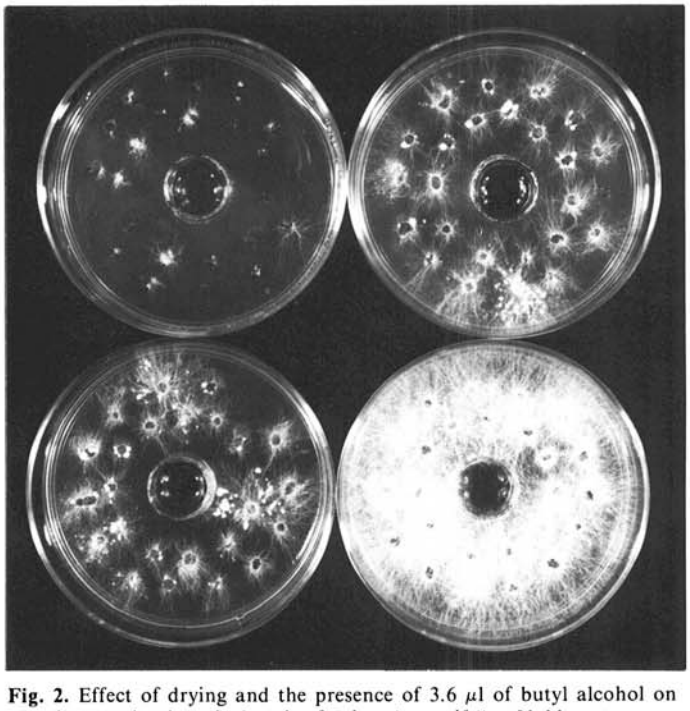

Fig. 2. Effect of drying and the presence of 3.6 μl of butyl alcohol on eruptive germination of sclerotia of *Sclerotium rolfsii* on Noble water agar. Upper row: nondried (left) and dried (right) sclerotia; vial in the center of the plate contains water. Lower row: nondried (left) and dried (right) sclerotia; vial in the center contains butyl alcohol. Photograph was taken after 72 hr of incubation at 28 C in the dark.

and medulla) were visible in sclerotia from all three sources (Fig. 3). Sclerotia grown on PDA had a poorly melanized rind, thinner cortex, and more medullary tissue than sclerotia produced in the laboratory on oats or those from soil. The soil-produced sclerotia had a slightly darker rind, which was generally not uniform in thickness (Fig. 3c). Sclerotia grown on oats were more uniform in shape and similar structurally to those from soil, except for a slightly thinner rind and thicker cortical layer. A membrane-like material on the sclerotial surface (20) was observed only on sclerotia from PDA cultures.

DISCUSSION

Many of the alcohols that stimulated sclerotial germination of *S. rolfsii* in our study are known constituents of plant tissue distillates (3,7); they may be released from tissues by autocatalytic reactions. Dried and remoistened tissues were reported to evolve greater amounts of volatiles than nondried fresh, or partially decomposed, tissues (5,6). In aged seeds (13,14) and in wood pieces (11), the major source of volatiles were shown to be fatty acid peroxidation products. While enzymatic degradation of pectic compounds in plant cell walls by pectinmethylesterase was proposed as the mechanism by which methanol was released from peanut tissue (5), enzymatic and/or microbial activity was not considered to contribute significantly to the release of volatile compounds from alfalfa tissue, since autoclaved or propylene oxide-sterilized hay (20) and fresh hay (18) behaved similarly to nontreated and cured hay, respectively. Detectable amounts of methanol also were emitted from autoclaved peanut hay (5). Owens et al (18) reported that methanol and ethanol, both constituents of alfalfa hay, were less stimulatory to fungal growth than alcohols containing three to six carbon atoms. Of the alcohols tested in our study, methanol was the least stimulatory to sclerotial germination. At concentrations of 0.5, 0.7, 1, 1.33, and 3.0% (1, 1.4, 2, 2.7, and 6.0 µl per dish), germination on soil of soil-produced sclerotia was 38, 54, 58, 59, and 12%, respectively (*unpublished*). Previous investigators (5,23) reported, however, that methanol significantly increased sclerotial germination of *S. rolfsii*. The highest germination (85 and 100%) was obtained with 1.0 and 1.33% solutions, respectively (23). With the exception of octanol, other alcohols and numerous other compounds also tested in vitro were less stimulatory (5). These results are difficult to interpret or directly compare with our findings, however, since the compounds were tested at varying concentrations (not equimolar) and under different experimental conditions. It is possible that the level used (about 2.9 mg) (5) may not have been within the range stimulatory for *S. rolfsii*. In our study, isopropanol (1.5%) and butanol (1.8%) stimulated vigorous germination of nondried and dried sclerotia and increased the mycelial growth rate, but no significant differences in dry weight were recorded. Although it was suggested that volatile compounds may be utilized as a nutrient or carbon source (5,23,25), this seems unlikely in view of the low concentrations (nanograms per milliliter) and brief (15 min) exposure times that are stimulatory (11,13,15). In one study (25), the increase in growth reported (0.1–2.0 mg) seems too small to permit comparison. For methanol to be utilized as a carbon source, enzymes capable of oxidation of C_1 compounds are requisite (8). Although they are present in bacteria (8), there is no evidence that these enzymes are produced by *S. rolfsii*. Fries (11) has proposed that volatile compounds could stimulate fungal growth by altering the levels, or increasing the efficiency of, certain enzymes; by altering membrane structure; or by acting as metabolic regulators (11). Increased leakage of amino compounds and carbohydrates from nondried sclerotia of *S. rolfsii* following exposure to volatile compounds from alfalfa hay could have resulted from subtle changes in membrane permeability or enzyme activity. The importance of volatile compounds as stimulants of sclerotial germination and mycelial growth in soil under field conditions, however, is unknown. Undoubtedly, the final concentrations in the microenvironment around sclerotia would be much lower than those shown to be stimulatory in our study. The hypothesis that these volatile compounds (in particular methanol) could in part serve as a stimulus for initiating epidemics of southern stem rot of peanuts (5), or explain the wide host range of *S. rolfsii* (5) requires further investigation.

The germinability responses of sclerotia from oats, PDA, and soil to methanol, butanol, and NaClO were different. While these treatments enhanced germination of nondried and dried sclerotia from oats, germination of dried sclerotia from PDA was reduced, especially by NaClO. Linderman and Gilbert (16) reported that sclerotia produced on PDA were more sensitive to extended NaClO treatment than those from soil, although volatile compounds and brief NaClO treatment increased germination of both types of sclerotia. The differences observed in this study in the thickness and degree of melanization of the rind and thickness of the cortical layer between sclerotia from PDA and soil could in part account for the differential sensitivity to NaClO. Although sclerotia from soil were stimulated by methanol and butanol in our study, highest germination both on agar and soil was observed following NaClO treatment. This increase most likely resulted from the elimination of contaminating microorganisms from the sclerotial surface. The hypothesis (16) that the increase in germination of culture- or soil-produced sclerotia by NaClO

TABLE 3. Eruptive germination of laboratory- and soil-produced sclerotia of *Sclerotium rolfsii* on unsterilized field soil and on 1% Noble water agar following exposure to two alcohols, NaClO, or drying

		Germination of sclerotia from various sources (%)[z]					
		Laboratory-produced				Soil-produced	
		PDA cultures		Oat cultures		Greenhouse	Field
Treatment[y]	Assay substrate	Nondried	Dried	Nondried	Dried	Dried	Dried
Water (control)	Soil	11	70	12	74	21	16
	Agar	29	98	27	100	56	24
Methanol	Soil	19	74	23	79	55	32
	Agar	35	77	46	100	68	36
Butanol	Soil	31	80	71	98	69	59
	Agar	65	82	96	100	82	69
NaClO	Soil	21	52	75	86	73	60
	Agar	46	64	82	100	86	74
LSD ($P = 0.05$)		4	3	4	3	6	7

[y] 0.2 ml of an aqueous solution (1.2%) of methanol or butanol was added to a 1-cm-high × 0.5-cm-wide glass vial and placed in the center of a 60 × 15-mm petri dish. For the NaClO treatment, sclerotia were immersed for 3 min in a 0.5% NaClO solution and then rinsed in water.

[z] Percentage of germination on the surface of unsterilized field soil and on 1% Noble water agar was determined after 3 days of incubation at 28 C in the dark. The data are the means of three separate experiments, with four replicates of 25 sclerotia each.

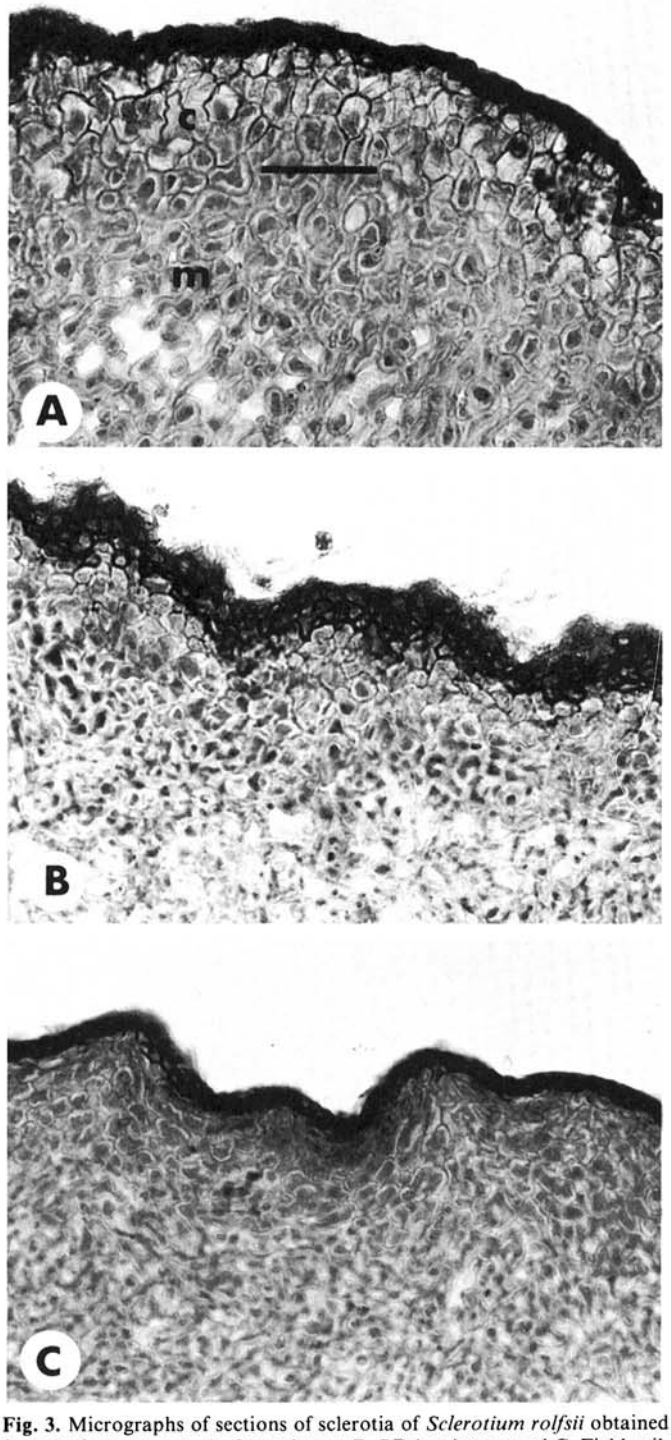

Fig. 3. Micrographs of sections of sclerotia of *Sclerotium rolfsii* obtained from various sources. **A,** Oat cultures; **B,** PDA cultures; and **C,** Field soil. Horizontal bar represents approximate delimitation of the cortex (c) from the medulla (m). Sections were stained with crystal violet-fast green-orange G.

treatment was the indirect result of changes in microbial activity in soil by enhanced nutrient exudation from the treated sclerotia (16) is inconsistent with the observation that germination, especially of sclerotia from soil, also was enhanced on sterile agar. This suggests a more direct effect of NaClO on the sclerotia themselves, similar to that observed with volatile compounds (20). Since germination of soil sclerotia was consistently low and quite variable in this and in other studies (5,6,16), even following NaClO treatment, they may be less desirable for research purposes than sclerotia produced on oats in the laboratory; these appear to be similar in structure and behavior to those from soil and germinate consistently. We agree with the advocacy of previous investigators (6,16,20) that the use of sclerotia produced on PDA should be avoided.

Considerable leakage of amino compounds and carbohydrates was detected from sclerotia during eruptive germination and the initial dry weight was reduced to about 65% after 72 hr. Saito (24) reported that dry weight of sclerotia of *Sclerotinia sclerotiorum* was similarly reduced during carpogenic germination. A 33% weight loss was associated with conversion of stored β-1,3 glucans to soluble products by glucanases (24). In *Saprolegnia ferax* (4), breaking of oospore dormancy also was associated with the hydrolysis and mobilization of glucans in the inner spore wall by glucanases. An increase in the level or activity of glucanases during eruptive germination of *S. rolfsii* could account for the observed increase in leakage and dry weight loss. Changes in plasma membrane permeability following drying and NaClO treatment (20) or exposure to alcohols, all of which have been reported to stimulate eruptive germination (20), also could result in mobilization of stored glucans through interaction with previously compartmentalized glucanases. Dry weight loss and leakage were reduced by adding glucose, indicating that reserve materials within the sclerotium were not solubilized, and the sclerotia underwent hyphal germination. Nutrients in PDA and impurities in Bacto water agar may be the reason why eruptive germination was inhibited in a previous study (20) when sclerotia were incubated on these media. Addition to Noble agar of carbon sources such as glucose that could be metabolized and used for growth similarly inhibited eruptive germination. Nonmetabolizable carbon sources such as mannitol (1) or 2-deoxyglucose (*unpublished*), amino acids, and sclerotial exudates, had no effect. The concentration of glucose in the sclerotial exudate ($\simeq$5mM [26]) may have been below the threshold for inhibition. These results indicate that substrates low in exogenous nutrients, such as acid-washed quartz sand (20), should be employed when assaying for eruptive germination. The inhibition by carbon sources may occur through catabolite repression (9,19,28) (reduction in rate of enzyme synthesis) or catabolite inhibition (inhibition of enzyme activity) (19). Catabolite repression in bacteria (9,19) and of zoosporangial formation in *Phytophthora capsici* (29) was reversed by adding cyclic AMP. In *P. megasperma* f. sp. *medicaginis*, inhibition of oospore germination by glucose was partially reversed by Na acetate and asparagine (10). None of these compounds, however, significantly increased the percentage of eruptive sclerotial germination in the presence of glucose. Thus, although exogenous nutrients are not required for eruptive germination and subsequent infection of host tissue by *S. rolfsii* (21), their presence in the environment near sclerotia may inhibit eruptive germination and promote hyphal germination, ensuring that reserve materials within the sclerotium are conserved. This has important implications concerning the infection behavior of *S. rolfsii*; in soil containing soluble nutrients and root exudates at high concentrations, infections may be initiated by sclerotia germinating hyphally if mycelial contact is made with senescent or dead tissue (21). At low nutrient concentrations, or in their absence, eruptively germinating sclerotia can infect without a food base (21). If volatile compounds evolved from plant tissues also are present at stimulatory levels, the distances over which mycelia can grow are increased considerably; furthermore, contact with the source of volatiles is not necessary (21). These factors, and interactions among them, could potentially influence the extent of disease caused by *S. rolfsii*.

LITERATURE CITED

1. Abeygunawardena, D. V. W., and Wood, R. K. S. 1957. Factors affecting the germination of sclerotia and mycelial growth of *Sclerotium rolfsii* Sacc. Trans. Br. Mycol. Soc. 40:221-231.
2. Allen, R. N., and Newhook, F. J. 1973. Chemotaxis of zoospores of *Phytophthora cinnamoni* to ethanol in capillaries of soil pore dimensions. Trans. Br. Mycol. Soc. 61:287-302.
3. Arai, S., Koyanagi, O., and Fujimaki, M. 1967. Studies on flavor components in soybean. Part IV. Volatile neutral compounds. Agric. Biol. Chem. 31:868-873.
4. Beakes, G. W. 1980. Electron microscopic study of oospore maturation and germination in an emasculate isolate of *Saprolegnia ferax*. 2. Wall

differentiation. Can. J. Bot. 58:195-208.
5. Beute, M. K., and Rodriguez-Kabana, R. 1979. Effect of volatile compounds from remoistened plant tissues on growth and germination of sclerotia of *Sclerotium rolfsii.* Phytopathology 69:802-805.
6. Beute, M. K., and Rodriguez-Kabana, R. 1979. Effect of wetting and the presence of peanut tissues on germination of sclerotia of *Sclerotium rolfsii* produced in soil. Phytopathology 69:869-872.
7. Bondarovich, H. A., Giammarino, A. S., Renner, J. A., Shephard, F. W., Shingler, A. J., and Gianturco, M. A. 1967. Some aspects of the chemistry of tea. A contribution to the knowledge of the volatile constituents. J. Agric. Food. Chem. 15:36-47.
8. Colby, J., Dalton, H., and Whittenbury, R. 1979. Biological and biochemical aspects of microbial growth on C_1 compounds. Annu. Rev. Microbiol. 33:481-517.
9. Epstein, W., Rothman-Denes, L. B., and Hesse, J. 1975. Adenosine 3′,5′-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli.* Proc. Nat. Acad. Sci. USA. 72:2300-2304.
10. Förster, H., Ribeiro, O. K., and Erwin, D. C. 1983. Factors affecting oospore germination of *Phytophthora megasperma* f. sp. *medicaginis.* Phytopathology 73:442-448.
11. Fries, N. 1973. Effects of volatile organic compounds on the growth and development of fungi. Trans. Br. Mycol. Soc. 60:1-21.
12. Gilbert, R. G., and Griebel, G. E. 1969. The influence of volatile substances from alfalfa on *Verticillium dahliae* in soil. Phytopathology 59:1400-1403.
13 Harman, G. E., Mattick, L. R., Nash, G., and Nedrow, B. L. 1980. Stimulation of fungal spore germination and inhibition of sporulation in fungal vegetative thalli by fatty acids and their volatile peroxidation products. Can. J. Bot. 58:1541-1547.
14. Harman, G. E., Nedrow, B., and Nash, G. 1978. Stimulation of fungal spore germination by volatiles from aged seeds. Can. J. Bot. 56:2124-2127.
15. Linderman, R. G., and Gilbert, R. G. 1969. Stimulation of *Sclerotium rolfsii* in soil by volatile components of alfalfa hay. Phytopathology 59:1366-1372.
16. Linderman, R. G., and Gilbert, R. G. 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.
17. Mowe, G., King, B., and Senn, S. J. 1983. Tropic responses of fungi to wood volatiles. J. Gen. Microbiol. 129:779-784.
18. Owens, L. D., Gilbert, R. G., Griebel, G. E., and Menzies, J. D. 1969. Identification of plant volatiles that stimulate microbial respiration and growth in soil. Phytopathology 59:1468-1472.
19. Paigen, K., and Williams, B. 1970. Catabolite repression and other control mechanisms in carbohydrate utilization. Pages 251-324 in: Advances in Microbial Research. Vol. 4. A. H. Rose and J. F. Wilkinson, eds. Academic Press, London and New York. 353 pp.
20. Punja, Z. K., and Grogan, R. G. 1981. Eruptive germination of sclerotia of *Sclerotium rolfsii.* Phytopathology 71:1092-1099.
21. Punja, Z. K., and Grogan, R. G. 1981. Mycelial growth and infection without a food base by eruptively germinating sclerotia of *Sclerotium rolfsii.* Phytopathology 71:1099-1103.
22. Punja, Z. K., and Jenkins, S. F. 1984. Influence of temperature, moisture, modified gaseous atmosphere, and depth in soil on eruptive sclerotial germination of *Sclerotium rolfsii.* Phytopathology 74:749-754.
23. Rodriguez-Kabana, R., Beute, M. K., and Backman, P. A. 1980. A method for estimating numbers of viable sclerotia of *Sclerotium rolfsii* in soil. Phytopathology 70:917-919.
24. Saito, I. 1977. Studies on the maturation and germination of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary, a causal fungus of bean stem rot. Hokkaido Pref. Agric. Exp. Stn. Rep. 26. 106 pp.
25. Schenck, S., and Stotzky, G. 1975. Effect on microorganisms of volatile compounds released from germinating seeds. Can. J. Microbiol. 21:1622-1634.
26. Smith, A. M. 1972. Nutrient leakage promotes biological control of dried sclerotia of *Sclerotium rolfsii* Sacc. Soil. Biol. Biochem. 4:125-129.
27. Stotzky, G., and Schenck, S. 1976. Observations on organic volatiles from germinating seeds and seedlings. Am. J. Bot. 63:798-805.
28. Turian, G. 1973. Induction of conidium formation in *Neurospora* by lifting of catabolite repression. J. Gen. Microbiol. 79:347-350.
29. Yoshikawa, M., and Masago, H. 1977. Effect of cyclic AMP on catabolite-repressed zoosporangial formation in *Phytophthora capsici.* Can. J. Bot. 55:840-843.