# Eruptive Germination of Sclerotia of *Sclerotium rolfsii*

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## ABSTRACT

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Eruptive germination, a previously undescribed form of germination of sclerotia of *Sclerotium rolfsii*, was characterized by plug(s) of mycelium erupting through the sclerotial rind, utilization of internal stored materials leaving an empty sclerotial rind, and by production of secondary sclerotia. This form of germination was observed on unsterilized field soil, on acid-washed and sterilized quartz sand, and on 1% Noble water agar. On 1% Bacto water agar and on Difco PDA, however, hyphal germination predominated. This form of germination was characterized by growth of individual hyphal strands from the surface of the sclerotium. Sclerotia of four isolates from 2-wk- to 8-mo-old sterile cultures in vitro and from artificially or naturally infested field soil germinated eruptively following drying for 7–10 hr at 15–20% RH or exposure to volatiles from various dried and remoistened plant tissues (hay). Washing sclerotia for 1–5 hr under running water, treating for 3 min with 0.5% NaOCl, or puncturing the sclerotial rind each promoted eruptive germination to a lesser extent. The rate, but not the percent, of eruptive germination was increased if sclerotia were washed in water before drying or exposure to hay. Sclerotia conditioned to germinate eruptively exuded greater amounts of amino compounds and carbohydrates than nonconditioned sclerotia.

Investigations on germination of sclerotia of *Sclerotium rolfsii* in vitro have shown that factors such as drying (6,27,28,32), exposure to volatile materials (primarily methanol, acetaldehyde, isobutyraldehyde, and isovaleraldehyde) from dried and remoistened plant tissues (hay) (4,5,17–19,22), treatment with NaOCl (6,19,32), and puncturing of the sclerotial rind (7) promoted germination. Percent germination in these studies was determined by incubation on various nutrient media (1,7,9,14), filter paper (6,17), glass filters (5), sand (27), or field soil (4,5,17–19,27,28). However, the type of germination (hyphal or eruptive) was not specified.

We have distinguished two forms of germination of sclerotia of *S. rolfsii*, hyphal and eruptive, and have shown that sclerotia germinating eruptively do not require a food base for infection of

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susceptible host tissues (23,24).

The objectives of this study were to determine the conditions that enhance or are required for competency of sclerotia to germinate eruptively, and to determine the effect of various germination assay substrates on eruptive germination.

## MATERIALS AND METHODS

**Source of isolates.** *S. rolfsii* isolates 1001, 1002, 1115, and 2672 obtained in California from bean, sugar beet, tomato, and bentgrass, respectively, were used in this study. In addition, isolate 120 from blue lupine and isolate 122 from peanut (isolates Scr-41 and Scr-48, respectively, courtesy of D. K. Bell, University of Georgia, Tifton 31794) were included in some experiments. Isolates were hyphal-tipped and maintained on potato-dextrose agar (PDA, pH 6.8 after autoclaving) at 24–26 C and a 14-hr photoperiod under cool-white fluorescent lights (General Electric, 20-watt, approximately 1,500 lux).

**Production of sclerotia.** *Culture-produced sclerotia.* Sclerotia used in most experiments were produced in 500-ml Erlenmeyer flasks containing 100 g of oat seeds, 50 ml distilled water, and 100 ml of 1% Difco Bacto water agar. The medium was prepared by adding the water and melted agar to oats that had been autoclaved on two successive days at 121 C and 1.05 kg/cm$^2$ pressure. The flasks were inoculated with two 1-cm-diameter agar disks cut from the edge of a 10-day-old PDA culture and maintained in the laboratory (~20 C) with occasional shaking by hand. Abundant sclerotia, all apparently mature, were formed after 2 mo of incubation.

*Soil-produced sclerotia.* In some experiments, sclerotia from three additional sources were compared with sclerotia produced in vitro; produced on tomato plant parts in 15-cm-diameter pots containing either unsterilized field soil (F-1) or 1:1 mixtures of soil and pasteurized UC mix (3), according to the method of Linderman and Gilbert (19); sieved from soil collected from the rhizosphere of infected sugar beet plants in Sutter County, CA, by using the method of Leach (16); grown on oats for 8 days at 20 C as above and then incorporated into unsterilized field soil (F-1) in 25-cm-diameter pots and incubated for an additional 2 wk at 30 C according to the method of Beute and Rodríguez-Kabana (5). The F-1 soil collected in Sutter County, CA, from rhizospheres of infected sugar beet plants, was sieved through a 0.85-mm (20-mesh) screen to remove large pieces of organic matter and naturally occurring sclerotia, and stored in plastic bags in the laboratory until used. The soil was a Tyndall very fine sandy loam with the following characteristics: pH 6.8, organic matter content <1%, conductivity 330 mmhos, and moisture-holding capacity at saturation 250 ml/kg of dry soil.

**Testing sclerotial germination.** Sclerotial germination was determined in most experiments by incubation on moistened acid-washed and sterilized quartz sand (Ottawa Silica Co., Ottawa, IL) which was sieved through a 0.71-mm (24-mesh) screen. Approximately 25 cm$^3$ was added to each 100 × 25-mm glass petri dish. The pH of the sand (2:1 mixture of sand and 0.01 M $CaCl_2$) was reduced from 8.6 to 5.5 by three washes with 0.1 N HCl and three rinses with distilled water. In some experiments, unsterilized F-1 field soil, 1% Difco Bacto water agar and 1% Difco Noble water agar (Difco Laboratories, Detroit, MI 48232) were used as the assay substrate. In all experiments, eight replicates of 25 sclerotia were incubated in petri dishes containing the various substrates at 27 ± 0.2 C and in the dark. Germination was assessed with the unaided eye within 72 hr. The percent germination reported pertains only to eruptive mycelial germination. All experiments were repeated at least twice.

**Effect of age and drying of sclerotia on eruptive germination.** Cultures of *S. rolfsii* were grown in 100 × 25-mm petri dishes containing 30 ml of Difco PDA at 27 C and a 14-hr photoperiod under cool-white fluorescent lights. Sclerotia, retrieved at weekly intervals starting at 2 wk and continuing until 10 wk after inoculation, were rinsed in water for 3 min and germination on quartz sand was tested either directly or after drying for 20 hr over $CaCl_2$ in a desiccator at 30 C.

**Effect of fluctuating ambient relative humidity on sclerotium formation on PDA and eruptive germination.** Cultures of *S. rolfsii* grown in petri dishes on PDA for 2, 4, and 6 days (as described above) were incubated in a Laminar airflow chamber (Laminar Flow Inc., Ivyland, PA 18974) with filtered air at 15–20% relative humidity (RH) blowing over them. Petri lids were removed for 0, 2, 4, 6, and 8 hr daily for five consecutive days. Excessive drying of the agar was prevented by daily addition of sterile distilled water to two 1-cm wells cut at the outer edges of each colony. After 5 days, sclerotial initials had formed and cultures were grown for an additional 14 days. At this time, the numbers of sclerotia formed and their ability to germinate on quartz sand were assessed. Sclerotia were tested either without additional drying or after drying for 20 hr over $CaCl_2$ in a desiccator at 30 C.

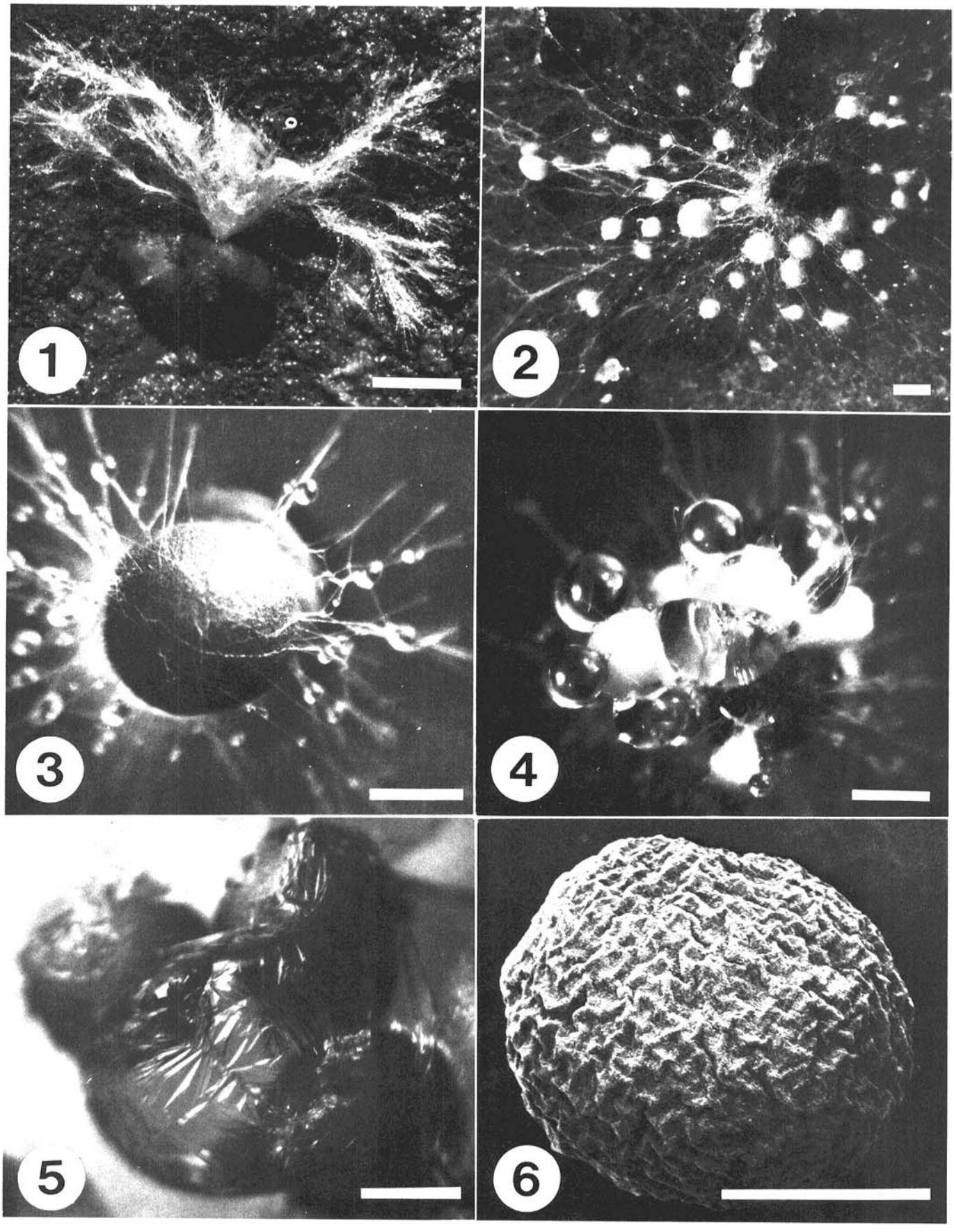
**Effect of treatments of the rind on germination and dry weight loss of sclerotia.** *Eruptive germination on various substrates.* Samples of sclerotia from in vitro oat cultures or those produced in soil were subjected to the following treatments: no treatment; dried for 20 hr over $CaCl_2$ in a desiccator at 30 C; punctured with a sharp dissecting needle to a depth of ~0.5 mm; or soaked for 3 min in either a 0.5% NaOCl solution or in 10% $H_2O_2$ and rinsed in water for 3 min. Sclerotia were assayed for eruptive germination on quartz sand, 1% Bacto water agar or 1% Noble water agar.

*Dry weight loss and nutrient leakage.* Five samples of sclerotia from in vitro oat cultures were subjected to the four treatments listed above. Leakage of water-soluble materials from treated sclerotia was determined by using ninhydrin reagent for total amino compounds (29) and anthrone reagent for total carbohydrates (36). One sample of sclerotia from each treatment was placed overnight in 5 ml of sterile distilled water at room temperature, and the ninhydrin and anthrone reagents were added separately to 1-ml samples of the solution; samples were processed following the procedures outlined by Smith (29) and Yemm and Willis (36), respectively. To determine the dry weight losses from washing, the four remaining samples from each treatment were washed under running water for 0, 1, 3, or 5 hr, dried for 24 hr in an oven at 105 C and weighed.

**Effect of drying on eruptive germination of sclerotia.** *Duration of drying.* Twenty 1-g (fresh wt) samples of sclerotia from oat cultures in vitro were dried for 0–10 hr either in forced air at 15–20% RH and 25 C or over $CaCl_2$ in a desiccator at 30 C. Two 1-g samples were retrieved every 30 min, weighed, and 200 sclerotia assayed for eruptive germination on quartz sand. The percent moisture remaining in the sclerotia after various times of drying, relative to the total moisture content of the sclerotia (determined by drying two 1-g samples at 105 C for 24 hr), was determined.

*Extent of drying.* To establish the minimum moisture tension to which sclerotia need to be dried to be capable of eruptive germination, 1-g samples of sclerotia from oat cultures in vitro were placed in 60 × 15-mm petri dish bottoms and incubated on inverted 50-ml beakers placed in tightly sealed 200-ml Mason jars containing saturated solutions of the following salts: $BaCl_2 \cdot 2H_2O$, KCl, $(NH_4)_2SO_4$, NaCl, $NH_4NO_3$, $Mg(NO_3)_2 \cdot 6H_2O$ and $MgCl_2 \cdot 2H_2O$. These saturated solutions provided RH values at 25 C of 90, 84, 81, 75, 62, 53, and 33%, respectively (25). Jars containing sclerotia were immersed almost completely in a water bath maintained at 25 ± 0.2 C. Sclerotia were removed after 2 wk, weighed to determine moisture content and 200 sclerotia were assayed for germination on quartz sand. In a subsequent experiment, RH values between 98 and 90% were tested using saturated salt solutions of $CuSO_4 \cdot 5H_2O$), $KH_2PO_4$, $Na_2HPO_4 \cdot 12H_2O$, $NH_4H_2PO_4$, $K_2HPO_4$ and $ZnSO_4 \cdot 7H_2O$, which provided RH values at 20 C of 98, 96.5, 95, 93, 92, and 90%, respectively (33,35).

**Effect of washing and drying treatments on rate of sclerotial germination.** Sclerotia from oat cultures in vitro were subjected to the following treatments: (i) no treatment; (ii) dried for 5–24 hr either in forced air at 15–20% RH and 25 C in a laminar airflow chamber or at 0–10% RH over $CaCl_2$ in a desiccator at 30 C; (iii) not dried, but washed for 1, 3, or 5 hr in water flowing at a rate of 1 L/min into 50-ml beakers covered with cheesecloth; (iv) washed as in (iii) then dried as in (ii); (v) dried as in (ii) then washed as in (iii).




**Figs. 1–6.** Sclerotia of *Sclerotium rolfsii* at various stages of germination and development. **1,** Eruptive germination of dried sclerotium incubated for 72 hr on moistened unsterilized field soil showing plug of mycelium bursting through the sclerotial rind. **2,** Secondary sclerotial production 8 days after eruptive germination on acid-washed and sterilized quartz sand. **3,** Hyphal germination on 1% Bacto water agar, showing individual hyphal strands emanating from the surface of the sclerotium. **4,** Droplets of liquid on developing sclerotia growing on PDA. **5,** Membranous material surrounding droplet of liquid on almost-matured sclerotia growing on PDA. **6,** Scanning electron micrograph of a dried sclerotium showing intact rind. Scale bar represents 1 mm.

Percent eruptive germination on quartz sand was assessed at intervals from 12–65 hr.

**Scanning electron microscopy of sclerotia.** Sclerotia from treatments (ii) and (iv) described above were attached to 12-mm aluminum stubs using double-sided (Double-Stick) Scotch tape, sputter-coated with gold (using argon as the inert gas), and examined with a Cambridge stereoscan electron microscope.

**Effect of volatiles from dried and remoistened plant tissues (hay) on sclerotial germination.** Two samples each of sclerotia either from in vitro oat cultures or produced in soil, were subjected to the following treatments: washed for 3 hr in water; soaked for 3 min in a 0.5% NaOCl solution; dried for 10 hr at 15–20% RH; and untreated. The effects of remoistened plant tissues (hay) on eruptive germination was tested on one sample by exposing the sclerotia to the hay for 48 hr; the other sample served as the unexposed control. The plant tissues tested were alfalfa (*Medicago sativa* L.), peanut (*Arachis hypogaea* L.) sugar beet (*Beta vulgaris* L.) and bentgrass (*Agrostis tenuis* L.); leaves and stems of greenhouse-grown plants were dried for 24 hr at 105 C, ground in a Wiley mill, and sieved through a 0.50-mm (32-mesh) screen.

Two methods were used to test the effects of hay on sclerotial germination: 0.05 g was placed in a 1-cm-wide × 0.5-cm-deep acid-washed glass vial, moistened with three drops of sterile distilled water, and placed in the center of a 60 × 15-mm petri dish containing either 1% Noble water agar, moistened quartz sand, or unsterilized field soil (F-1) wetted to field capacity (~ −1/3 bar) 48 hr prior to use. Sclerotia were placed around the vial. Controls in separate dishes had only sterile distilled water in the vials; or 2 g of hay was placed in a 60 × 15-mm petri dish bottom, moistened with 5 ml of sterile distilled water and placed in the bottom of a 9.2-L desiccator; open petri dishes containing sclerotia on various substrates were placed in the desiccator on a supporting screen. Control plates were placed in a desiccator not containing hay. This method was adapted from the studies by Beute and Rodríguez-Kabana (4,5).

In subsequent experiments, autoclaved hay (20 min at 121 C and 1.05 kg/cm$^2$ pressure) and hay sterilized with propylene oxide were compared to untreated hay for effects on sclerotial germination. In addition, untreated hay to which was added activated charcoal (Darco® G-60, Matheson Coleman and Bell, Norwood, OH 45212) at a 10% (w/w) rate was tested.

## RESULTS

Eruptive mycelial germination of sclerotia of *S. rolfsii* was observed on sterilized quartz sand, 1% Noble water agar, and on unsterilized field soil, but it occurred infrequently on 1% Bacto water agar or PDA. Eruptively germinating sclerotia produced a plug of mycelium that burst through the sclerotial rind (Fig. 1). Internal contents were utilized during germination and the sclerotium collapsed when pressed gently with a dissecting needle. Secondary sclerotia were produced consistently when sclerotia were germinated on a substrate lacking nutrients (Fig. 2). In contrast, hyphal germination was observed most frequently on 1% Bacto water agar and on PDA, and occasionally on quartz sand, 1% Noble water agar, and on field soil. It was characterized by growth of individual hyphal strands from the surface of the sclerotium (Fig. 3). Unless provided with an exogenous nutrient source, growth from hyphal germination was barely perceptible.

**Effect of age and drying of sclerotia on eruptive germination.** Germination on quartz sand of undried sclerotia from 1-wk-old PDA cultures was about 3% and percent germination progressively increased to 42% for sclerotia from 10-wk-old PDA cultures (Fig. 7). If sclerotia from 2- to 10-wk-old cultures were dried before testing, however, all samples germinated 94–100%.

**Effect of fluctuating ambient relative humidity on sclerotial formation on PDA and eruptive germination.** Exposure of 2-, 4-, and 6-day-old PDA cultures of *S. rolfsii* to air at 15–20% RH for 2, 4, 6, and 8 hr daily for 5 days progressively decreased the capacity of cultures to produce sclerotia. At all exposure times from 2 to 8 hr, more sclerotia were produced in the 4- and 6-day-old cultures than in the 2-day-old cultures. Amber-colored droplets of liquid consistently associated with developing sclerotia of *S. rolfsii* in closed dishes (Fig. 4) were not produced if the lids were removed for any period of time. Droplets produced in closed dishes were surrounded by a film of saclike membranous material (Fig. 5). Germination of sclerotia previously exposed to ambient RH for 2 to 8 hr was consistently low on quartz sand, ranging from 0 to 9%. When these sclerotia were dried, however, germination of all samples increased to about 85%.

**Effect of treatments of the rind on germination and dry weight loss of sclerotia.** *Eruptive germination on various substrates.* Percent eruptive germination was affected by treatments of sclerotia and the assay substrate (Table 1). Variability among isolates was most pronounced when undried sclerotia were used; percent eruptive germination of all isolates tested ranged from 6 to 48%. Drying of sclerotia increased germination to 99–100% and reduced the variability. After 8 mo in oat culture, sclerotia consistently germinated 85–100% if dried. Storage of dried

TABLE 1. Effect of treatments of sclerotia of *Sclerotium rolfsii* on eruptive germination on three substrates

| Sclerotial treatment[x] | Eruptive germination (%):[w] Quartz sand | 1% Noble water agar | 1% Bacto water agar |
|---|---|---|---|
| None | 31[y] d[z] | 37[y] d | 1 c |
| Dried over $CaCl_2$ for 20 hr | 100 a | 100 a | 14 a |
| Undried, punctured | 45 c | 52 c | 2 c |
| Undried, 0.5% NaOCl for 3 min | 56 b | 64 b | 5 b |
| LSD ($P = 0.01$) | 6.1 | 5.8 | 2.7 |

[w] Germination of sclerotia on the three substrates was assessed after 72 hr incubation at 27 C. Data are the means of eight replications of 25 sclerotia each; the experiment was repeated twice.

[x] Sclerotia of four isolates from 2-mo-old oat cultures in vitro were subjected to the respective treatments prior to incubation on the substrates. Data for soil-produced sclerotia also tested are not presented.

[y] Variability among isolates in precent germination was significant ($P = 0.05$) only for untreated sclerotia; range of germination values was 6–48%.

[z] Means in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

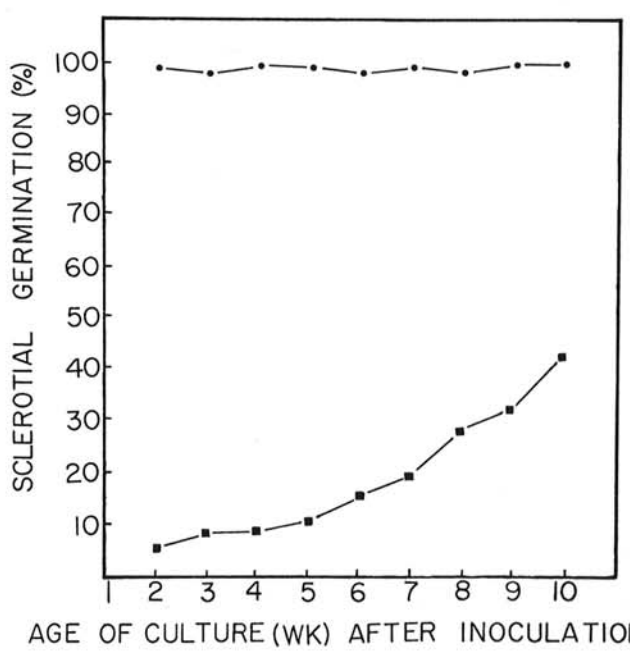



**Fig. 7.** Effect of age and drying of sclerotia of *Sclerotium rolfsii* on germination. Undried sclerotia (■——■) from PDA cultures were compared at weekly intervals from 2 to 10 wk with dried sclerotia (•——•). Germination was assessed after incubation for 72 hr on quartz sand at 27 C. Points represent means of three separate experiments, with eight replicates in each experiment.

sclerotia over $CaCl_2$ in desiccators for 10 days to 2 wk, however, reduced germination to 20–30%. Treatment with NaOCl and puncturing were not as effective in promoting eruptive germination as drying (Table 1). Soaking sclerotia for 3 min in 10% $H_2O_2$ also did not increase germination. On 1% Noble water agar and on quartz sand, germination of dried sclerotia was primarily eruptive; however, overall percent germination on Noble agar was slightly higher than on quartz sand. Although total (hyphal and eruptive) germination on 1% Bacto water agar approached 100%, the hyphal form predominated on this medium, and eruptive germination was <15%.

Sclerotia produced in field soil and those recovered from naturally infested soil did not differ greatly in general responses to the treatments as compared with sclerotia produced on oats in vitro. Percent germination on quartz sand before drying ranged from 19 to 28%; after drying, it was 62–70%. The sclerotia produced in soil were darker and the rind was bleached less by NaOCl treatment, but germination was increased following NaOCl treatment to about the same extent as for sclerotia produced in vitro. Percent germination of sclerotia from soil was consistently less than that of sclerotia from oats apparently due to frequent contamination by species of *Penicillium* and *Trichoderma*.

*Dry weight loss and nutrient leakage.* Leakage of amino compounds and carbohydrates from sclerotia was greatly increased by drying for 20 hr over $CaCl_2$ (Table 2). Sclerotia that had been punctured with a sharp needle, but not dried, leaked only small amounts of amino compounds and carbohydrates, and dry weight losses after 3 and 5 hr of washing were only 3.5 and 4.8%, respectively. Untreated sclerotia did not leak any detectable amino compounds and carbohydrates, although a 3.9% loss in dry weight was recorded after 5 hr of washing (Table 2). Soaking sclerotia for 3 min in 0.5% NaOCl increased leakage and dry weight losses of 7.4 and 9.7% were recorded following 3 and 5 hr of washing, respectively.

**Effect of drying on eruptive germination of sclerotia.** *Duration of drying.* Moisture loss from sclerotia dried either in air at 15–20% RH or over $CaCl_2$ was very rapid; only 8% of the total moisture (fresh weight) remained after 7 hr (Fig. 8). Although sclerotia had been routinely dried for 10–20 hr over $CaCl_2$ prior to use, such extensive drying was unnecessary. Drying sclerotia in air at 15–20% RH for periods as short as 1.5 hr removed 44% of the moisture and increased eruptive germination from ~28 to ~97% (Fig. 8). The total moisture content of undried sclerotia was about 62%. Germination on quartz sand of sclerotia dried for 7 hr at 15–20% RH was vigorous, and after about 8 days, four to five secondary sclerotia were produced from each germinating sclerotium (Fig. 2). These secondary sclerotia, 3 wk after their formation on quartz sand, were capable of eruptive germination only if initially dried; they also produced smaller tertiary sclerotia following germination. On natural field soil, secondary sclerotial formation was greatly reduced, averaging about one for every two germinating parent sclerotia.

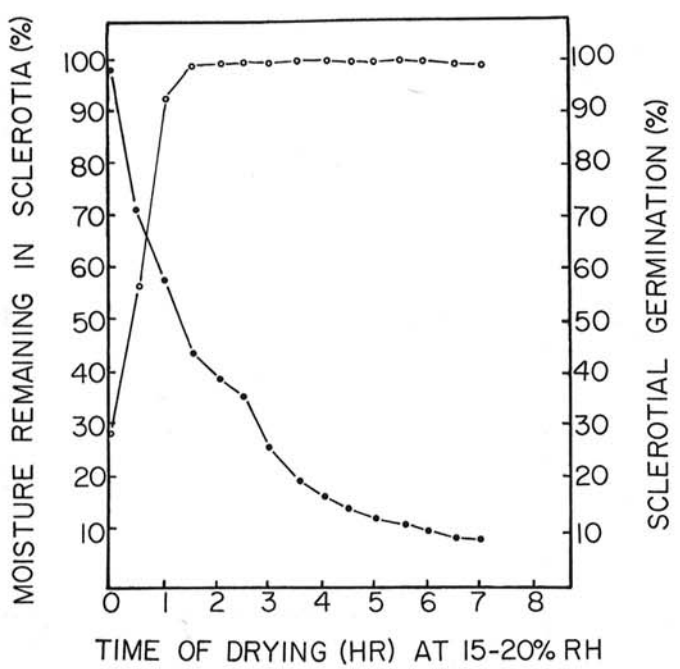



**Fig. 8.** Relationship between time of drying of sclerotia of *Sclerotium rolfsii* at 15–20% RH and moisture content (•——•) and eruptive germination (o——o). Sclerotia from 2-mo-old in vitro oat cultures were dried in a laminar airflow chamber at 15–20% RH and retrieved at 30-min intervals for moisture determination and percent germination. Points represent means of three separate experiments, with eight replicates in each experiment.

*Extent of drying.* Sclerotia incubated for 2 wk over saturated salt solutions at RH ranging from 33 to 90% lost 92 to 79% of their total moisture content and germinated eruptively from 99 to 86%, respectively. Sclerotia dried at RH between 90 and 95% germinated ~65–85%. Moisture loss from sclerotia incubated at ambient RH greater than 95% was negligible, and ability to germinate eruptively was not increased in comparison with the undried control.

**Effect of washing and drying treatments on rate of sclerotial germination.** Undried sclerotia washed in water for 1, 3, or 5 hr germinated more rapidly and total percent eruptive germination on quartz sand was higher than for unwashed, nondried sclerotia (Fig. 9). Similarly, washing prior to drying of sclerotia promoted more rapid germination, but total percent germination was not affected. Drying followed by washing for similar periods of time, however, significantly delayed onset of germination, but percent germination after 65 hr of incubation was similar (Fig. 9). Drying sclerotia either in air at 15–20% RH or over $CaCl_2$ was equally effective in promoting eruptive germination.

**Scanning electron microscopy of sclerotia.** Sclerotia from oat culture that were washed for 3 hr in water and dried over $CaCl_2$, when examined under the scanning electron microscope (up to ×1,100) did not have cracks in the sclerotial rind (Fig. 6). Dried sclerotia appeared darker and shriveled while undried sclerotia retained a smooth surface. Sclerotia that had not been washed prior to drying had a membranous material adhering to the surface that resembled the film surrounding droplets of liquid on developing sclerotia in closed petri dishes (Fig. 5).

**Effect of volatiles from dried and remoistened plant tissues (hay) on sclerotial germination.** Volatiles from all four plant tissues were stimulatory to eruptive germination, whether tested in petri dishes or in desiccators. Soil-produced sclerotia, undried or dried, responded similarly to volatiles from the hays as sclerotia produced on oats in vitro; all isolates of *S. rolfsii* tested also responded to the

TABLE 2. Dry weight loss and leakage of amino compounds and carbohydrates during washing of sclerotia of *Sclerotium rolfsii* subjected to different treatments

| Sclerotial treatment[w] | Dry weight loss (%) after washing for various times in water[x] | | | Leakage of amino compounds and carbohydrates[y] |
|---|---|---|---|---|
| | 1 hr | 3 hr | 5 hr | |
| None | 0.8 c[z] | 2.9 c | 3.9 c | – |
| Dried over $CaCl_2$ for 20 hr | 6.8 a | 15.3 a | 25.0 a | +++ |
| Undried, punctured | 0.7 c | 3.5 c | 4.8 c | + |
| Undried, 0.5% NaOCl for 3 min | 2.7 b | 7.4 b | 9.7 b | ++ |
| LSD ($P = 0.01$) | 0.5 | 1.0 | 4.8 | |

[w] Sclerotia of four isolates from 2-mo-old oat cultures in vitro were subjected to the respective treatments and then washed in tap water for various times.

[x] Based on weights after drying for 24 hr at 105 C. Data are the means of two replications and the experiment was repeated twice.

[y] Presence of amino compounds and carbohydrates was detected using the ninhydrin and anthrone reagents, respectively; amount of leakage was estimated on the basis of relative intensities of color development.

[z] Means in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

volatiles. Alfalfa hay gave most consistent results but peanut, sugar beet, and bentgrass were only slightly less effective. Although sclerotia were routinely exposed to volatiles from the moistened hays for 48 hr, subsequent experiments showed that a minimum of 12 hr of exposure was sufficient to promote eruptive germination. Undried sclerotia in the presence of hay germinated as well as dried sclerotia without hay both on 1% Noble water agar and on quartz sand, but on field soil, percent germination of undried sclerotia in the presence of hay was greater than that of dried sclerotia without hay (Table 3). Undried sclerotia not exposed to the hays germinated from 6 to 48%, depending on the isolate, the age of the culture, and the substrate used; exposure to hay increased this to 88–92%. Bleached or washed sclerotia germinated better than untreated sclerotia on all substrates, and exposure to volatiles from hay promoted even greater germination of these treated sclerotia. Percent germination of dried sclerotia was not significantly increased by exposure to volatiles from hay except on field soil where germination was increased from 78 to 100%; a similar increase was obtained when dried sclerotia were incubated on autoclaved, but not on unsterilized field soil. On all substrates tested, mycelial growth from dried sclerotia was apparently much more vigorous when hay was present. Autoclaved and propylene oxide-sterilized hays also were stimulatory to germination. Addition of activated charcoal only slightly reduced the stimulatory effect; germination of undried sclerotia on 1% Noble water agar was reduced from 92 to 79% but mycelial growth was still vigorous.

## DISCUSSION

The eruptive form of sclerotial germination has been described for *Sclerotinia minor* (2) and *Sclerotium cepivorum* (10), but this is the first detailed report for *S. rolfsii*. From examination of various published photographs, however, it appears that other workers have recorded eruptive germination both in *S. rolfsii* and in *S. delphinii* without being aware of it (5,15,17–19,27). Sclerotia germinating in an eruptive manner can do so only once because stored energy reserves are utilized in growth of the mycelium (24). Reports of sclerotia germinating more than once therefore can pertain only to the hyphal form of germination (14–16). Also, reports of germination of sclerotia on nutrient media (1,7,9,14) are difficult to interpret because the type of germination (eruptive vs hyphal) was not specified. We observed, however, that eruptive germination was inhibited when competent sclerotia were incubated on PDA or 1% Bacto water agar, but not when sclerotia were incubated on the more purified Noble water agar. We therefore routinely test for eruptive germination in the absence of nutrients by using either acid-washed quartz sand, 1% Noble water agar, or field soil sieved to remove particles of organic matter. Preliminary studies have shown that various nutrient sources such as 50 mM glucose and lactose when added to 1% Noble water agar inhibited eruptive germination and only hyphal germination was observed (*unpublished*). Formation of secondary sclerotia following germination has been occasionally reported for *S. rolfsii* (17,27) and for *S. delphinii* (15); we observed consistent formation of secondary sclerotia by competent sclerotia of *S. rolfsii* that germinated eruptively on a substrate devoid of nutrients. Formation of secondary sclerotia has been reported for *S. minor* (2) and *S. cepivorum* (10), but they usually are produced less consistently.

Droplets of liquid have been observed associated with developing sclerotia of various fungi in sterile culture (13,30) and a membranous material surrounding these droplets similar to that reported here has been observed for *Sclerotinia sclerotiorum* and *Claviceps purpurea* in addition to *S. rolfsii* (11–13). We attach no biological significance to this membranous material that some workers have referred to as a cuticle or skin (8,30,34). It may result from the polymerization of various compounds in the exudate that form a membranous or viscous surface film. Washing sclerotia removes this material and may account for the 3.9% loss in dry weight recorded when undried sclerotia were washed.

We did not detect major differences between sclerotia produced on oats in vitro and sclerotia produced in soil by either of two methods (5,19) in their general response to factors affecting competency for eruptive germination. Although sclerotia formed

TABLE 3. Eruptive germination of variously treated sclerotia of *Sclerotium rolfsii* on three substrates in the presence or absence of volatiles from moistened hay

| Sclerotial treatment[w] | Hay volatiles provided[x] | Germination on various substrates (%)[v] 1% Noble water agar | Quartz sand | Field soil |
|---|---|---|---|---|
| None | − | 38[y] e[z] | 29 f | 21 g |
| | + | 92 c | 88 c | 90 c |
| Nondried, washed 3 hr | − | 65 d | 62 d | 34 f |
| | + | 96 b | 9 b | 95 b |
| 0.5% NaOCl for 3 min | − | 67 d | 48 e | 54 e |
| | + | 100 a | 100 a | 100 a |
| Dried at 15–20% RH for 10 hr | − | 100 a | 98 a | 78 d |
| | + | 100 a | 100 a | 100 a |
| LSD ($P = 0.01$) | | 3.1 | 3.4 | 3.6 |

[v] Germination of sclerotia on the three substrates was recorded after 72-hr incubation at 27 C. Data are the means of eight replications of 25 sclerotia each; the experiment was repeated twice.

[w] Sclerotia of six isolates from 2-mo-old oat cultures were used.

[x] Hays tested included alfalfa, peanut, sugar beet and bentgrass; data are for alfalfa hay that was slightly more effective than the other materials; 0.05 g was added to a small glass vial placed in the center of a petri dish or 2.0 g was placed in a desiccator containing sclerotia.

[y] Variability among isolates in percent germination was significant ($P = 0.05$) only for untreated sclerotia; range of germination values was 6–48%.

[z] Means in a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

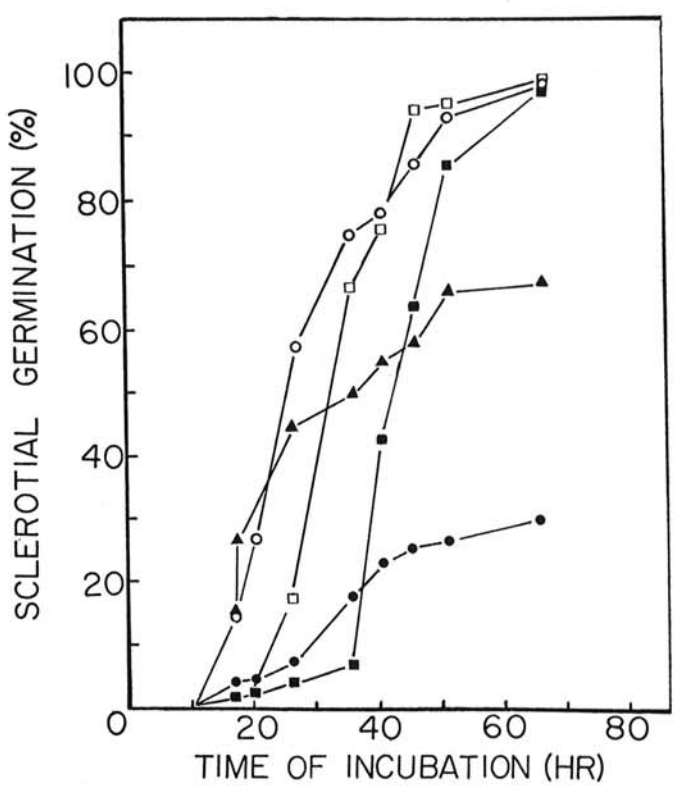



**Fig. 9.** Effect of washing and drying on rate of eruptive germination of sclerotia of *Sclerotium rolfsii*. Treatments were: no treatment (●——●); dried for 20 hr in air at 15-20% RH or over $CaCl_2$ (□——□); undried and washed in water for 1, 3, or 5 hr (▲——▲); washed, then dried (o——o); and dried, then washed (■——■). Points represent means of three separate experiments, with eight replicates in each experiment.

in the soil had a thicker and darker rind and the membranous material was absent, their responses to various factors investigated in this study were not altered. It may be these observed differences, however, that led Linderman (19) to conclude that sclerotia formed in soil appeared to be structurally and/or physiologically different from culture-grown sclerotia. We agree with his advocacy of the use of soil sclerotia instead of PDA-produced sclerotia; however, sclerotia produced on a substrate such as oats appear to be physiologically similar to those formed in soil, with the added advantages of being more uniform in size and free from microbial contamination.

Eruptive germination was observed most consistently in this study following short periods of drying of sclerotia or exposure of undried sclerotia to volatiles from hay. Fluctuating ambient RH during in vitro formation of sclerotia was not sufficient to trigger competency for eruptive germination of sclerotia that matured afterwards; the drying requirement had to be imposed upon sclerotia that had already matured. Apparently, mature sclerotia have a very short, if any, period of dormancy; thus sclerotia from 2-wk-old cultures were capable of eruptive germination if first dried. Undried sclerotia germinated poorly, as previously reported by Smith (27,28).

Loss of moisture from sclerotia during drying was rapid, indicating that the rind is not an effective barrier to water loss (27,31); up to 92% of the total moisture was lost after drying for 7 hr at 15–20% RH. The moisture content of 62% for these sclerotia is similar to that reported elsewhere (31).

Bleaching, washing, and puncturing of undried sclerotia each increased compentency for eruptive germination, but to a much lesser extent than drying or exposure to volatiles. Bleaching sclerotia has been reported to promote germinability (19), and our results indicate that eruptive germination is increased with NaOCl treatment but not with $H_2O_2$. Linderman (19) suggested that bleaching may nullify an internal dormancy in the sclerotia. In addition, increased exudation of nutrients from bleached sclerotia was reported to increase microbial activity in soil which in turn stimulated sclerotia to germinate (18–20); however, it is not clear whether the reported germination was eruptive or hyphal. Washing of sclerotia has been reported to either inhibit germination (9) or to have no effect (5); we observed an increase both in total percent germination and in the rate of germination when undried sclerotia were washed, and an increase only in rate of germination when sclerotia were washed prior to drying.

The mechanism(s) by which the factors investigated in this study affect capability for eruptive germination is not known. Boswell (6) and Chet (7) suggested that drying of sclerotia caused cracks in the rind through which hyphae could emerge. Chet (7) observed that germination of sclerotia punctured with a sharp needle was more rapid, especially on water agar, and concluded that the rind acted as a barrier to uptake of nutrients. We did not observe cracks in the sclerotial rind following drying. Also, puncturing the rind was much less effective than drying for increasing competency for eruptive germination. The increased germination recorded by Chet (7) possibly was hyphal instead of eruptive. Thus, the sclerotial rind apparently is not the major factor (physical or physiological) limiting ability of sclerotia to germinate eruptively.

Sclerotia leak considerable amounts of amino compounds and carbohydrates at the onset of germination, and losses in dry weight of up to 11.6% have been reported (9,18,19,28). Most treatments that promoted eruptive germination in this study also increased leakage from sclerotia. It is possible that drying and other treatments may alter cellular membrane permeability, thereby increasing leakage of materials including potentially inhibitory substances. Washing untreated sclerotia, however, promoted eruptive germination without substantial nutrient leakage or loss in dry weight, while puncturing the sclerotial rind, which promoted germination to a lesser extent than washing, resulted in slightly greater dry weight loss (Table 2). Attempts to increase leakage and promote germination by treating sclerotia for 5–20 min in a 0.1% solution of nystatin, dimethyl sulfoxide, or Triton X-100 (all known to disrupt membrane permeability) or mixtures of each at 37 C were not successful (*unpublished*). Materials leaked from sclerotia were not required for germination as suggested by Higgins (14), Smith (28), and Chet et al (9) and their removal by washing resulted only in a delay in onset of germination.

Eruptive germination may be dependent upon increased activity of an enzyme(s) in the sclerotia, resulting in solubilization and increased leakage of stored food materials. In *Sclerotinia sclerotiorum*, for example, competency for carpogenic germination is correlated with increased activity of $\beta$-1,3 glucanase and a decrease in dry weight of sclerotia (26). The enzyme converts stored $\beta$-1,3 glucans in sclerotia to soluble and readily utilizable products (26). We have not investigated this possibility for *S. rolfsii*.

Volatiles from hay may affect capability for germination of sclerotia either directly or indirectly by affecting soil microorganisms in the vicinity of the sclerotia, as reported by Linderman (17,20). Beute and Rodriguez-Kabana (4,5) also have shown that volatiles can act directly on sclerotia. Exposure of soil to the volatiles for 7 days, however, did not affect germination of untreated sclerotia subsequently placed on the soil surface (4); similarly, we did not observe a change in percent germination (24) although Linderman (18,20) showed that sclerotial germination could be drastically reduced, and attributed the reduction to a buildup of antagonistic microorganisms, primarily bacteria. An increase in germination was observed in Linderman's studies, however, when sclerotia and soil were exposed simultaneously to volatiles (18,20). This increase may not be due solely to a reduction in the antagonistic and/or to an increase in stimulatory microorganisms. Undried sclerotia were stimulated to germinate in this study by exposure to moistened hay in the absence of microorganisms on sterilized quartz sand and on 1% Noble water agar, indicating a direct mechanism of action on the sclerotia. However, the importance of microorganisms should not be excluded as an increase in germination of dried sclerotia on field soil from 78 to 100% by exposure to volatiles, an increase that also was obtained by autoclaving field soil, suggests that fungistasis imposed by antagonistic microflora stimulated by exudates from dried sclerotia (18,21) may have been alleviated by the addition of hay. Whether volatiles provide an energy source for growth of soil microflora or *S. rolfsii* was not determined.

Under natural field conditions, it is probable that a combination of factors could act to trigger eruptive germination of sclerotia, and results from this study indicate that certain combinations could greatly increase percent germination. For example, dried sclerotia subsequently exposed to volatiles germinate better than undried sclerotia also exposed to the volatiles, on a variety of substrates. The significance of eruptive germination in nature and its epidemiological importance are discussed elsewhere (24).

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