Influence of Temperature, Moisture, Modified Gaseous Atmosphere, and Depth in Soil on Eruptive Sclerotial Germination of Sclerotium rolfsii

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ABSTRACT


Eruptive sclerotial germination of Sclerotium rolfsii on Noble water agar or on the surface of unsterilized field soil was 80–100% at 21–30 °C, at \( \phi_r \) between -2.5 and -10 bars, and at \( \phi_w \) between 0 and -1 bar. Germination was not greatly reduced by CO\(_2\) levels in the range of 0.5–9%, \( O_2 \) levels between 15 and 20.5%, or \( CH_4 \) concentrations in the range of 1–60 mg/ml. Sclerotial germination was nil at 9–12 °C, at \( \phi_r \), < -60 bars, at \( \phi_w \), < -10 bars (in a course sandy loam) or < -15 bars (in a fine sandy loam), and at CO\(_2\) and \( O_2 \) levels of > 20% and < 3%, respectively. Burial of sclerotia in moistened unsterile or autoclaved field soil at depths of > 2.5 cm reduced germination and no sclerotia germinated at depths of > 7 cm. This inhibition apparently was not the result of lack of aeration and may have been due in part to the direct or indirect effects of pressure imposed physically on the sclerotia by soil at the greater depths. Placing metal weights over sclerotia on the soil surface inhibited germination, and exudation of amino compounds and carbohydrates from these sclerotia was much greater than leakage from sclerotia without the simulated pressure from the weights. Reports of reduced survival of sclerotia deeper in soil could in part be explained by this increase in leakage, which enhanced colonization by soil microorganisms.

Additional key words: water potential.

Sclerotia of the soilborne plant pathogen Sclerotium rolfsii Sacc. (teleomorph: Atthelia rolfsii (Curzi) Tu & Kimbrough) are the principal means by which the fungus survives in the absence of host tissue. These sclerotia can germinate either eruptively or hyphally, depending on environmental conditions before and during the onset of germination (25). Eruptive germination may be distinguished from hyphal germination by the presence of dense mycelial growth that originates from within the sclerotium, leaving an empty sclerotial ring (25). The infection behavior and extent of mycelial growth of S. rolfsii differ greatly depending on the form of germination (26), and have a significant bearing on the epidemiology of diseases caused by this pathogen (16).

Previous investigators studying the effects of environmental factors on sclerotial germination have not distinguished between the eruptive and hyphal forms of germination (1,3,18,22). Results from these studies indicate, however, that optimal germination (most likely hyphal) occurred at 25–30 °C (1,18), in soils adjusted to moisture-holding capacities (MHC) between 25 and 75% (1), and when sclerotia were at or near the soil surface (1,6,11). A reduction in germination of sclerotia buried deeper in soil was attributed to the depletion of \( O_2 \) and/or buildup of CO\(_2\) (1,18). The results from studies on the effects of aeration on mycelial growth (linear growth and dry weight) in culture are conflicting. Some investigators (19) reported that CO\(_2\) levels above 10% were inhibitory, whereas others (12,15,23) found that high CO\(_2\) or low \( O_2 \) had little effect. The direct effects of CO\(_2\) or \( O_2 \) on sclerotial germination have not been studied. Ethylene was reported to induce germination (31) or have no effect (3). The disparities among these studies may be due in part to differences in methodology and need to be resolved.

Reports on the relationships of soil texture and soil moisture with disease caused by S. rolfsii indicated that disease incidence was greater in light sandy soils than in silt or clay soils (2,9,35) and greater at MHC of 50–75% than at saturation (28). The effects of soil moisture (expressed as matric and osmotic potentials) and soil type on sclerotial germination have not been reported, however.

The objectives of this study were to determine the effects of temperature, soil moisture (both the osmotic \( \psi_s \) and matric \( \psi_m \) components), gaseous atmospheres (CO\(_2\), \( O_2 \), and \( CH_4 \), and depth of burial in soil on eruptive sclerotial germination of S. rolfsii.

MATERIALS AND METHODS

Isolates, production of sclerotia, and assessment of sclerotial germination. S. rolfsii isolates 1003, 1120, and 2672 obtained in California from sunflower, bean, and bentgrass, respectively, and isolate 159 from sorghum in North Carolina (courtesy of S. Gurkin, North Carolina State University) were used in this study. Sclerotia for all experiments were obtained from oat cultures (100 g of oat seeds, 30 ml of distilled water, and 90 ml of 1.5% Difco-Bacto water agar, prepared as described by Punja and Grogan [25], which were grown at 24–28 °C and a 12-hr/day photoperiod under cool-white fluorescent lights (General Electric, 20W). Sclerotia from 2- to 6-mo-old cultures were dried for 10–20 hr at 15–20% relative humidity or over CaCl\(_2\) in a desiccator before use to induce eruptive germination (25). In most experiments, germination was assayed on 1% Difco Noble water agar and on the surface of two unsterilized field soils. Both soils (F-1 from the rhizosphere of infected sugar beet plants in Sutter County, CA, and G-1 from Johnston County, NC) were air-dried, sieved through a 1.18-mm (14-mesh) screen, and stored in plastic bags in the laboratory until used. The F-1 soil was a fine sandy loam (sand-silt-clay, 63:20:17) with pH 5.8, organic matter (OM) content < 1%, and MHC at saturation of 25 ml/100 g of soil. The G-1 soil was a coarse sandy loam (77:16:7) with pH 5.6, OM content about 1%, and MHC of 18.5 ml/100 g of soil. The soils were moistened to field capacity (about -1/3 bar) with distilled water 48 hr before use. About 6 cm

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of soil or 10 ml of Noble agar was added to petri dishes (60 × 15
mm). In most experiments, four replicate dishes containing 25
sclerotia each were used; germination was assessed visually after
72–96 hr of incubation at 28 C in the dark. Each experiment was
repeated at least twice. The data presented are the means of the
replicates and repetitions of each experiment.

Temperature. Petri dishes with sclerotia were placed in
incubators maintained at constant temperatures ranging from 9 to
36 C, at increments of 3 C.

Solute water potential. The basal medium employed was 1%
Noble water agar (pH after autoclaving 5.8). The solute potential
(φs) was adjusted to values between −2.5 to −98 bars (Fig. 1) by
adding appropriate amounts of either CaCl2, H2O2, NaCl, or
sucrose as outlined by Robinson and Stokes (29). After autoclaving
for 15 min at 121 C and 1.05 kg/cm2 pressure, sterile distilled water
was added to the flasks to compensate for any losses in volume
during autoclaving. Ten milliliters of osmotically adjusted agar
was poured into each petri dish; dishes with sclerotia were incubated in
sealed plastic bags.

Matric water potential. Germination of sclerotia at matric water
potentials (ψm) ranging from 0 to −15 bars (Fig. 2) was assayed on
F-1 and G-1 soils. Sieved, air-dried soil was spread over ceramic
pressure plates and saturated with distilled water. Rubber rings (50
× 10 mm) were inserted into the soil to facilitate subsequent removal
of subsamples. The soil was left on the plates overnight, then
subjected to various suction for −3 to 7 days to obtain the desired
ψm. Each disk of soil (50 mm in diameter) was inoculated with 25
sclerotia as described previously. The dishes were then placed in
the dark for 5 days. The disks were then transferred to autoclaved
soil in some vials and sealed with a rubber stopper. Gas samples
were placed over the jars for 72 hr and the gas concentration
within the jars noted. In some experiment, the air was forced
into the pipette and through the soil to determine the effect
on sclerotial germination.

Effect of pressure. Stainless steel cylinders 37.5 mm in diameter
were cut to varying heights (from 5 to 30 mm, at increments of 5
mm). The weights of these cylinders (ranging from 45 to 270 g) were
determined and used to calculate the pressure (weight per unit of
area) exerted over an area (1 × 1 cm) of soil. These pressures ranged
from 0.004 to 0.024 kg/cm2. By comparison, the pressure imposed by
a 70-mm-high column of moistened F-1 field soil (contained in a
glass vial) 23 mm in diameter and weighing 58 g was calculated to be
about 0.014 kg/cm2. The metal weights were placed over sclerotia
distributed on filter paper or on the surface of a 2-mm-layer of
moistened field soil contained in petri dishes, in a Bürchner funnel,
or on a sieve (28-mesh). Germination of sclerotia around and under
the weights was rated after 72 hr. In some experiments, the effect of
enhancing gaseous exchange under these weights on germination of
sclerotia was determined by forcing air up through the bottom of a
Bürchner funnel. The effect of pressure from the weights on the
extent of leakage of amino compounds and carbohydrates from
both dried and nondried sclerotia was determined using the
ninhydrin and anthrone reagents, respectively (25). Sclerotia were
incubated on moistened filter paper disks for 18 hr with a weight
over them or were left untreated. The sclerotia were then removed
and the disks were air-dried before treatment with the respective
reagents (25).

RESULTS

Temperature. Eruptive germination of sclerotia on 1% Noble
agar and on F-1 field soil was nil at 9 and 12 C, 50–60% at 15–18 C,
and 80–100% at 21–30 C. At 33 and 36 C, the percentages of
germination were 85 and 56, respectively. Rate and vigor of
germination were reduced at temperatures below 21 C and were
highest at 27–30 C. The isolates varied slightly in their temperature
optima, which ranged from 26 to 30 C.

Solute water potential. The response of sclerotia to φs
influenced by the osmotic as well as the specific isolate. All isolates
were slightly more tolerant of NaCl and sucrose than CaCl2 (Fig. 1):
isolates 2622 and bentgrass were most tolerant to the three osmotica
tested. In general, maximum germination (85–100%) occurred at ψs
between −2.5 and −10 bars. With decreasing ψs, from −10 to −40
bars, the percentage of germination declined to about 10–20%. Below
−60 bars, germination was nil (Fig. 1).

Matric water potential. The germination response of sclerotia at
similar ψm values differed with the two soil types tested (Fig. 2). In a
cosmatically sandy loam (MHC of 18.5%), the percentage of germination
decreased almost linearly with an increase in moisture tension from
saturating. At −3 bars, germination was about 54% and no
sclerotia germinated at ψm below −10 bars. In contrast, in a fine
sandy loam (MHC of 25%), germination declined gradually with a
increase in ψm, at −3 bars, germination was about 88% and at −15
bars, about 22% (Fig. 2). The moisture content of the samples did
not change significantly during the 72-hr incubation period.

Modified gaseous atmosphere. Carbon dioxide. The germinability response of sclerotia to increasing CO2

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concentrations was similar on Noble agar and on field soil; the overall percentage of germination was higher on agar (Fig. 3). Germination was not greatly reduced from that of the control (0.5% CO₂) at CO₂ levels in the range of 0.5–1.9%. As the CO₂ concentration was increased above 9%, however, germination was markedly reduced but was 12–18% even at 19.8% CO₂ (Fig. 3). Linear mycelial growth was considerably more sensitive to increases in CO₂; it was greatly reduced at CO₂ levels above 1% and was only 38% of that in the air control at 9% CO₂ (Fig. 3).

**Oxygen.** The response of sclerotia to decreasing O₂ concentrations varied with the assay substrate. On Noble agar, there was a gradual but steady decline in percentage of germination with decreasing O₂ (Fig. 4). Germination was reduced to about 50% of that of the control (20.5% O₂) at 7% O₂. On field soil, the decrease in germination was much more marked than on Noble agar at O₂ levels below 13%. A 50% reduction in germination was observed at about 11% O₂ (Fig. 4). Linear mycelial growth on soil was more sensitive to decreasing O₂ concentrations than sclerotial germination and was reduced by 50% at about 14% O₂ (Fig. 4).

**Ethylene.** Sclerotial germination and linear mycelial growth were not affected by C₂H₄ concentrations in the range of 1–40 μg/ml. There were no differences between the percentage of germination in the air control (0.03 μg/ml of C₂H₄) and in jars injected with various amounts of C₂H₄.

**Depth in soil.** Percentage of eruptive germination was highest at the surface of unsterile and autoclaved field soil; total germination was greater in autoclaved soil. Sclerotial germination was only slightly reduced at depths of 5–25 mm (Fig. 5). With an increase in depth below 25 mm, percentage of germination decreased gradually and was nil at depths >70 mm. Sclerotia buried in acid-washed and sterilized quartz sand showed a similar germination response to increasing depth (Z. K. Punja and S. F. Jenkins, unpublished). The two methods used in this study to test the effect of soil depth gave comparable results and all isolates behaved similarly. In the presence of volatile compounds from hay, germination was increased over the control only for sclerotia buried at depths of 5–40 mm; at greater depths, there was no difference in the percentages of sclerotial germination in soil with or without hay (Fig. 5).

The composition of gas samples from the bottom of vials containing 70 mm of soil was 17.8% O₂; 2.2% CO₂; 80% N₂. Passage

**Fig. 1.** Effect of solute water potential on eruptive germination of sclerotia of *Sclerotium rolfsii* on Noble water agar. Each point represents the mean of three separate experiments, each with four replicates. Germination was rated after 72 hr of incubation at 28°C. Four isolates were tested; the data presented are for isolate 1120. The general response of other isolates was similar.

**Fig. 2.** Moisture release curves for two soils and the corresponding percentage of eruptive germination of sclerotia of *Sclerotium rolfsii* at various soil water matric potentials. Each point represents the mean of four replicates.

**Fig. 3.** Effect of carbon dioxide concentration on eruptive germination (——) of sclerotia of *Sclerotium rolfsii* on Noble agar and field soil and linear mycelial growth (-----) on soil after 72 hr of incubation. Each point represents the mean of three separate experiments, each with four replicates. The CO₂ levels were monitored after 12 and 72 hr.
of air through pipettes inserted in the soil did not increase germination of sclerotia buried at depths of 50–70 mm. The viability (determined by surface-sterilization with NaOCl and incubation on water agar) of these ungerminated sclerotia was reduced from 100 to about 80% after a 7-day period. The microorganisms isolated most frequently from nonviable sclerotia were species of *Fusarium*, *Penicillium*, and *Trichoderma*.

**Effect of pressure.** Germination of sclerotia on filter paper or on the soil surface was completely inhibited by placing metal weights that imposed pressures greater than 0.012 kg/cm² over them (Fig. 6). Experiments conducted in Bückner funnels or over sieves to allow gaseous exchange to occur from below also gave similar results. Forcing air through the Bückner funnel did not alleviate the inhibition. When the weights were removed and the sclerotia on soil were incubated for an additional 7 days, they did not germinate and were colonized by microorganisms. Leakage of amino compounds and carbohydrates from dried and nondried sclerotia incubated under weights was about 2.2 times greater than leakage from comparable sclerotia without pressure from the weights.

**DISCUSSION**

The optimal temperature range for eruptive sclerotial germination of *S. rolfsii* on Noble agar and on unsterilized field soil is similar to that reported by others for linear growth and mycelial dry weight production in culture (1,11,17,18). In these studies, maximum growth occurred at 30 C and was nil at 8 and 40 C.

Previous studies on the effects of soil moisture on sclerotial germination and disease development have expressed moisture status as percentage of MHC (1,28). This expression of soil water content does not accurately reflect the adsorption and capillary forces in soil, which constitute *ψ*<sub>m</sub> (10), and also differs with soil type. Thus, these results are difficult to interpret. In this study, sclerotia of *S. rolfsii* germinated at *ψ*<sub>m</sub> as low as −7 bars but

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**Fig. 4.** Effect of oxygen concentration on eruptive germination (—) of sclerotia of *Sclerotium rolfsii* on Noble agar and field soil and linear mycelial growth (—) on soil after 72 hr of incubation. Each point represents the mean of three separate experiments, each with four replicates. The O<sub>2</sub> levels were monitored after 12 and 72 hr.

**Fig. 5.** Effect of depth of burial in unsterilized field soil in the absence or presence of dried and remoistened alfalfa hay on eruptive germination of sclerotia of *Sclerotium rolfsii*. Bars represent the means of three separate experiments, each with four replicates. Germination was rated after 5 days of incubation at 28 C.

**Fig. 6.** Effect of imposing unidirectional pressure in the form of a stainless steel weight on germination of sclerotia of *Sclerotium rolfsii* on unsterilized field soil. A, Germination of sclerotia around the weight. B, Inhibition of germination underneath the weight. Both photographs are of the same petri dish and were taken after 72 hr of incubation. Scale bar = 2 cm.
maximum germination occurred between 0 and 1 bar. Although the moisture requirements for mycelial growth and infection have not been determined, preliminary studies indicated that infections can occur from sclerotia germinating at $\psi_w$ of -1.3 and -0.5 bar (Z. K. Punja and S. F. Jenkins, unpublished). Sclerotial germination was less sensitive to decreasing $\psi_w$ than to decreasing $\psi_h$ and occurred at soil potentials as low as -30 bars. A similar differential sensitivity to $\psi_h$ and $\psi_h$ has been reported for other fungi (10).

Percentage of sclerotial germination at similar $\psi_m$ was greater in a fine sandy loam than in a coarser-textured soil. Lifshitz and Hancock (21) reported that increases in the propagule densities of _Pythium ultimum_ in soil at equal $\psi_m$ values were greater in a clay soil than in a sandy loam. It was suggested that increased nutrient availability at higher moisture contents could account in part for the difference between the two soils (21). Because extracellular scleriotics are not requisite for eruptive germination of sclerotia of _S. rolfsii_ (and may inhibit it) (25), the observed differences in the percentage of germination in the two soil types employed in this study at similar $\psi_m$ could possibly be due to other differences, such as in texture, water content, and/or composition of the soil microflora.

Percentage of germination was highest at the soil surface and was nil at depths greater than 7 cm both in unsterile and autoclaved soil. Volatile compounds from alfalfa hay increased germination of sclerotia only if they were in the upper 4 cm of soil; this is consistent with an earlier report (22). Numerous investigators have observed that sclerotia buried at various depths (ranging from 2 to 15 cm) did not germinate (1,6,11,33); this was attributed by some to inhibition by high CO$_2$ levels (1,18). However, the direct effects of CO$_2$ on germination were not established (1). Because extracellular sclerotiotics are not greatly reduced by CO$_2$ concentrations up to 9%, although linear mycelial growth on soil was affected. Griffith and Nair (15) reported that the linear growth rate on PDA was reduced by 50% only if CO$_2$ levels were >11.5%, and Mitchell and Mitchell (23) observed a 50% reduction in dry weight at 15% CO$_2$. In contrast, Kritzman et al (19) reported that linear growth and dry weight were increased at 1% CO$_2$ but were nil and 20% of the air control, respectively, at 12% CO$_2$. These measurements were made after a 10-hr exposure to CO$_2$ (19); the changes in growth after this short incubation period probably would not have been significant enough to justify comparisons. Most of the available information supports the contention of Griffith and Nair (15) and Coley-Smith and Cooke (7) that CO$_2$ accumulation could not account for the observed inhibition of sclerotial germination of _S. rolfsii_ in soil. Although the results of Rodriguez-Kabana (8.94) of CO$_2$ and addition of O$_2$ (by BaO$_2$) enhanced sclerotial germination on soil in the presence (but not absence) of plant tissue amendments, it is possible that these changes in O$_2$ and CO$_2$ could have indirectly enhanced germination by altering the composition of soil microflora that also are stimulated by volatiles from plant tissues (24). The response of fungi to CO$_2$ may also be influenced by the pH and composition of the assay medium (32). Dissolved CO$_2$ may under alkaline conditions be converted to the bicarbonate (HCO$_3^-$) ion, which is considerably more toxic to sclerotia of _S. rolfsii_ than CO$_2$ at lower pHs (27).

Reduction of O$_2$ was reported to have very little effect on linear growth (12,15) and dry weight (23) until the level was reduced to 4 and 1%, respectively. The effects of O$_2$:CO$_2$ mixtures were similar to those of CO$_2$ alone. Formation of sclerotal initials, however, was inhibited when the O$_2$ level was reduced to 15% or CO$_2$ increased above 4% (15). This could explain why sclerotia of _S. rolfsii_ commonly are formed at or near the soil surface (2) and are found primarily in the uppermost 15 cm of soil (20). In this study, sclerotial germination on agar and soil decreased gradually as O$_2$ levels were reduced, but the decline was much more pronounced on soil at O$_2$ levels below 13%. We attributed this difference (which was partially eliminated by sterilizing the soil) to increased abundance of microorganisms that were observed colonizing the ungerminated sclerotia at the low O$_2$ levels and that could have prevented their germination. Flados (12) reported similar observations and suggested that the occurrence of _S. rolfsii_ predominately near the soil surface could be the result of greater antagonism deeper in the soil.

When weights that imposed pressures calculated to be similar to those imposed by a column (6-7 cm high) of moist soil (about 0.012-0.014 kg/cm$^2$) were placed over sclerotia, germination on various assay substrates was completely inhibited. Increasing gaseous exchange to these sclerotia did not promote germination. Comparable soil pressures over deep buried sclerotia could possibly (either directly or indirectly) account for the inhibition of germination at depths of >7 cm observed in this and in previous studies (1,6,11,33). Sclerotia at these depths could also be more sensitive to changes in O$_2$ and CO$_2$ levels.

We are aware of the possibility that the unidirectional pressure imposed by weights in our system may not realistically reflect the multidirectional pressure impinging on sclerotia by the surrounding soil. These pressures would vary with texture (sand:silt:clay ratio) and moisture content of various substrates. For example, the pressure would be relatively high (about 0.009 $\psi_m$ units) in the presence of a thin layer of clay at the soil surface, and it could be negligible in the presence of a layer of sand. Furthermore, the pressure would be greatest where the soil is most disturbed. Similar considerations may apply to forces exerted by soil animal activity and root growth. It is possible that pressures generated by these activities are sufficient to prevent germination of buried sclerotia. Also, it is possible that pressures generated by these activities are insufficient to prevent the germination of a large number of sclerotia. It is possible that these forces are insufficient to prevent the germination of a large number of sclerotia. It is possible that these forces are insufficient to prevent the germination of a large number of sclerotia. It is possible that these forces are insufficient to prevent the germination of a large number of sclerotia. It is possible that these forces are insufficient to prevent the germination of a large number of sclerotia. It is possible that these forces are insufficient to prevent the germination of a large number of sclerotia.
thus depriving the fungus of a food base thought to be a requisite for infection (5,13). It appears more likely, however, that sclerotia that were turned under were inhibited from germinating (6,11) and were decomposed. It has been shown that dried sclerotia of *S. rolfsii* that germinated eruptively did not require a food base for infection (26). In dry or light-textured sandy soils, such infections may occur well below the soil surface (2,34), possibly because sclerotia in these soils were subjected to increased drying or reduced pressure, both of which would be expected to enhance germination. Under these conditions, the (competence) volume of soil (16) for infection by *S. rolfsii* may be increased.

**LITERATURE CITED**