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

Z. K. Punja and S. F. Jenkins

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

Journal Series Paper 9060 of the North Carolina Agricultural Research Service, Raleigh.

Mention of trade or company names does not imply endorsement by the Campbell Soup Company or the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.

We thank S. Gurkin for assistance with the gaseous composition study, K. Cassel and M. Saltveit for use of their facilities, and M. Williams for preparation of illustrations.

This research was supported in part by a grant to North Carolina State University from the Campbell Soup Company.

Accepted for publication 6 April 1984 (submitted for electronic processing).

ABSTRACT

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.

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 ψ_s between −2.5 and −10 bars, and at ψ_m 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 C_2H_4 concentrations in the range of 1–40 μg/ml. Sclerotial germination was nil at 9–12 C, at $\psi_s < -60$ bars, at $\psi_m < -10$ bars (in a coarse 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: *Athelia 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 rind (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 either inhibit 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 [ψ_s] and matric [ψ_m] components), gaseous atmospheres (CO_2, O_2, and C_2H_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, 50 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^3

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

© 1984 The American Phytopathological Society

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 (ψ_s) was adjusted to values between −2.5 to −58 bars (Fig. 1) by adding appropriate amounts of either $CaCl_2 \cdot 2H_2O$, NaCl, or sucrose as outlined by Robinson and Stokes (29). After autoclaving for 15 min at 121 C and 1.05 kg/cm^2 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 (ψ_m) 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 pressed into the soil to facilitate subsequent removal of subsamples. The soil was left on the plates overnight, then subjected to various suctions for 1–3 days to obtain the desired ψ_m. Each disk of soil (50 mm in diameter) was inoculated with 25 sclerotia that were gently pressed into the soil. The disks were placed in glass petri dishes (100 × 15 mm) that were then wrapped with Parafilm and sealed in cellophane bags. The moisture content of the two soils at each ψ_m was determined at the start of the experiment by drying at 105 C and the data were used to establish moisture-release curves. Moisture content readings also were taken at the end of the experiment.

Modified gaseous atmospheres. All experiments were conducted in sealed 3.8-L jars with tight-fitting screw-cap lids. Two sections (30 mm long × 2 mm wide) of a pipette were inserted through holes punched in the lids to provide inlet and outlet openings. The end of each pipette was sealed with a rubber stopper that could be pierced with a syringe to introduce or remove gas samples. For CO_2 experiments, a portion of the air in the jar was displaced through the outlet by 100% CO_2 flowing into the jar for a calculated time period at a rate of 100 or 200 ml/min. The level of CO_2 (in the range of 0.5–20.5%) in each jar was determined after 12 and 72 hr by analyzing gas samples on a Fisher gas partitioner 1200 chromatograph. For O_2 experiments, a portion of the air in sealed jars was displaced with 100% N_2 flowing at 400 ml/min; levels of O_2 in jars ranged from about 3.5 to 20.5%. Gas samples were analyzed after 12 and 72 hr. For C_2H_4 experiments, small volumes of 10,000 μg/ml of C_2H_4 were injected into sealed jars to achieve concentrations ranging from 1 to 40 μg/ml; the actual levels were monitored after 12 and 72 hr.

Sclerotial germination. Petri dishes with sclerotia on soil and Noble agar were placed on moistened paper towels in the jars, which were then sealed and adjusted to various concentrations of CO_2, O_2, or C_2H_4 as described before. The jars were incubated at room temperature (24–27 C) in the dark for 72 hr.

Linear mycelial growth. Petri dishes containing moistened soil were inoculated with a 1-cm-diameter mycelial plug taken from the margin of a 5-day-old PDA culture and incubated in the jars. Measurements of linear growth of the mycelium over the soil surface in the modified atmospheres were made after 72 hr.

Depth in soil. Two methods were employed: In one series of experiments, 15 sclerotia were placed at the bottom of a glass vial (93 × 23 mm) and covered with varying amounts of unsterile or autoclaved field soil, which was subsequently moistened to about −1/3 bar. Germination was assessed after 5 days by counting the colonies visible through the bottom of the vial. To determine if volatile compounds from dried and remoistened alfalfa hay (25) influenced germination of sclerotia at these depths, a vial 1 cm wide × 0.5 cm high containing 50 mg of hay was placed on the soil surface. Control vials received sterile distilled water.

In a second series of experiments, sclerotia contained in PVC cylinders (15 × 10 mm) fitted with a 0.5-mm wire-mesh bottom were placed in vials partially filled with a 20-mm depth of soil. Additional soil was added over the sclerotia to achieve various depths of burial. After 5 days, the sclerotia were retrieved by sieving and rated as having germinated if visible mycelium originated from the sclerotium and if the sclerotial rind collapsed when pressed gently with a dissecting needle (25). In both series of experiments, sclerotia also were incubated on the soil surface in each vial. The vials were sealed with a rubber stopper pierced with two 1-mm-diameter needles to allow for gaseous exchange. The soil depths tested ranged from 5 to 70 mm, at increments of 5 mm. To monitor the gaseous atmosphere within the vials during the experiment, a 10-cm section of a pipette (2 mm in diameter) was fully inserted in the soil in some vials and sealed with a rubber stopper. Gas samples were taken after 72 hr by piercing the stopper and drawing the air within the pipette into a syringe. In some experiments, 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/cm^2. 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/cm^2. 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 Büchner 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 Büchner 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 greatest 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 ψ_s was influenced by the osmotica as well as the specific isolate. All isolates were slightly more tolerant of NaCl and sucrose than $CaCl_2$ (Fig. 1); isolate 2672 from bentgrass was most tolerant to the three osmotica tested. In general, maximum germination (85–100%) occurred at ψ_s between −2.5 and −10 bars. With decreasing ψ_s 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 ψ_m values differed with the two soil types tested (Fig. 2). In a coarse sandy loam (MHC of 18.5%), the percentage of germination declined almost linearly with an increase in moisture tension from saturation. At −3 bars, germination was about 54% and no sclerotia germinated at ψ_m below −10 bars. In contrast, in a fine sandy loam (MHC of 25%), germination declined gradually with a decrease in ψ_m; 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 CO_2

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% CO_2) at CO_2 levels in the range of 0.5–9%. As the CO_2 concentration was increased above 9%, however, germination was markedly reduced but was 12–18% even at 19.8% CO_2 (Fig. 3). Linear mycelial growth was considerably more sensitive to increases in CO_2; it was greatly reduced at CO_2 levels above 1% and was only 38% of that in the air control at 9% CO_2 (Fig. 3).

Oxygen. The response of sclerotia to decreasing O_2 concentrations varied with the assay substrate. On Noble agar, there was a gradual but steady decline in percentage of germination with decreasing O_2 (Fig. 4). Germination was reduced to about 50% of that of the control (20.5% O_2) at 7% O_2. On field soil, the decrease in germination was much more marked than on Noble agar at O_2 levels below 13%. A 50% reduction in germination was observed at about 11% O_2 (Fig. 4). Linear mycelial growth on soil was more sensitive to decreasing O_2 concentrations than sclerotial germination and was reduced by 50% at about 14% O_2 (Fig. 4).

Ethylene. Sclerotial germination and linear mycelial growth were not affected by C_2H_4 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 C_2H_4) and in jars injected with various amounts of C_2H_4.

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% O_2:2.2% CO_2:80% N_2. Passage

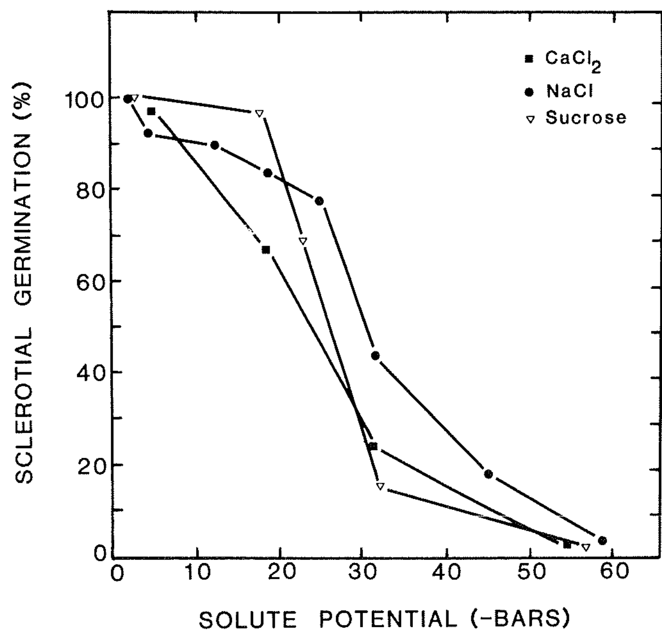


Fig. 1. Effect of solute water potential on eruptive germination of sclerotia of *Sclerotium rolfsii* on Noble water agar. Each point represents the mean of three separate experiments, each with four replicates. Germination was rated after 72 hr of incubation at 28 C. Four isolates were tested; the data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

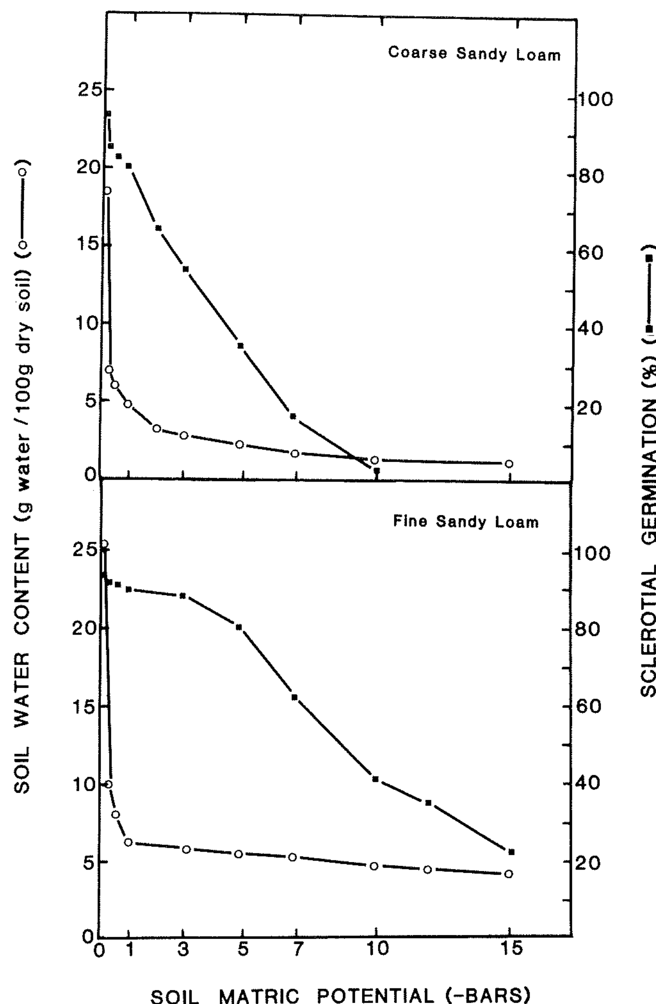


Fig. 2. Moisture release curves for two soils and the corresponding percentage of eruptive germination of sclerotia of *Sclerotium rolfsii* at various soil water matric potentials. Each point represents the mean of four replicates.

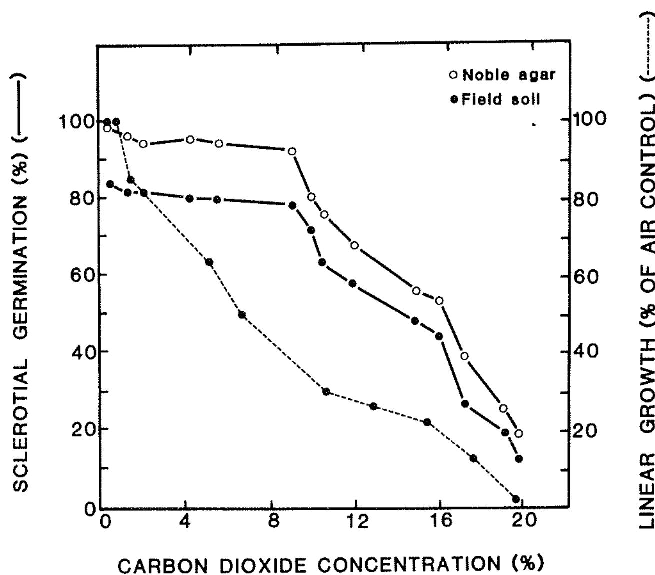


Fig. 3. Effect of carbon dioxide concentration on eruptive germination (——) of sclerotia of *Sclerotium rolfsii* on Noble agar and field soil and linear mycelial growth (----) on soil after 72 hr of incubation. Each point represents the mean of three separate experiments, each with four replicates. The CO_2 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/cm^2 over them (Fig. 6). Experiments conducted in Büchner funnels or over sieves to allow gaseous exchange to occur from below also gave similar results. Forcing air through the Büchner 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.

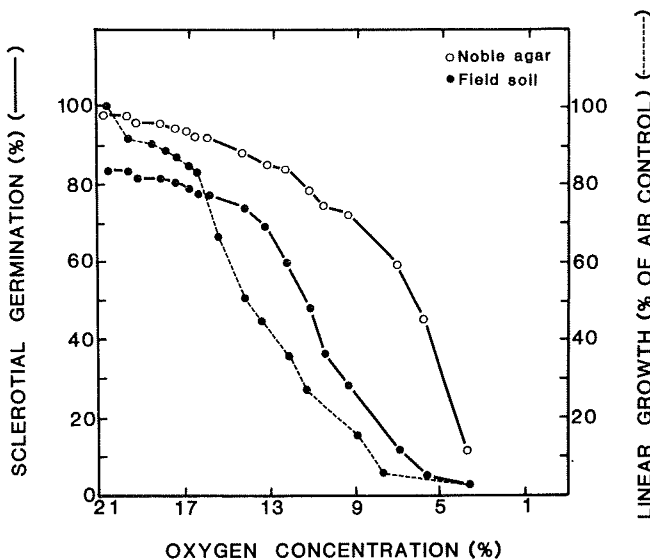


Fig. 4. Effect of oxygen concentration on eruptive germination (——) of sclerotia of *Sclerotium rolfsii* on Noble agar and field soil and linear mycelial growth (----) on soil after 72 hr of incubation. Each point represents the mean of three separate experiments, each with four replicates. The O_2 levels were monitored after 12 and 72 hr.

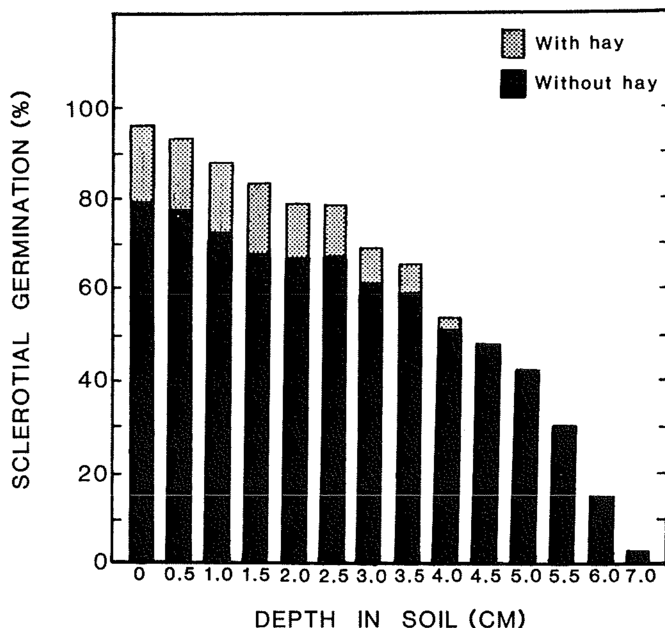
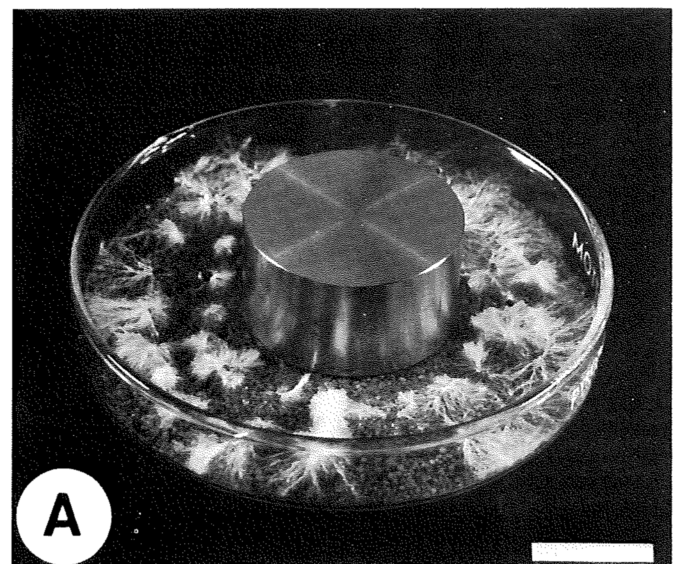


Fig. 5. Effect of depth of burial in unsterilized field soil in the absence or presence of dried and remoistened alfalfa hay on eruptive germination of sclerotia of *Sclerotium rolfsii*. Bars represent the means of three separate experiments, each with four replicates. Germination was rated after 5 days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

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_m (10), and also differs with soil type. Thus, these results are difficult to interpret. In this study, sclerotia of *S. rolfsii* germinated at ψ_m as low as −7 bars but



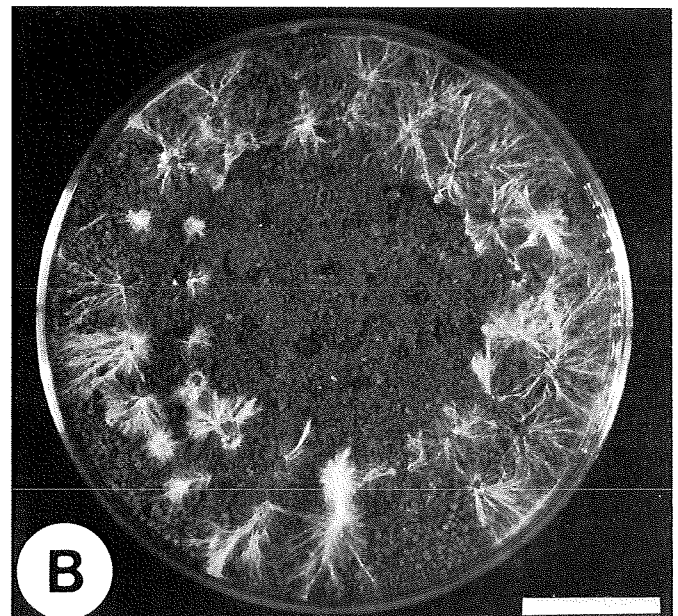


Fig. 6. Effect of imposing unidirectional pressure in the form of a stainless steel weight on germination of sclerotia of *Sclerotium rolfsii* on unsterilized field soil. **A,** Germination of sclerotia around the weight. **B,** Inhibition of germination underneath the weight. Both photographs are of 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 ψ_m of −1/3 and −0.5 bar (Z. K. Punja and S. F. Jenkins, *unpublished*). Sclerotial germination was less sensitive to decreasing ψ_s than to decreasing ψ_m and occurred at solute potentials as low as −30 bars. A similar differential sensitivity to ψ_s and ψ_m has been reported for other fungi (10).

Percentage of sclerotial germination at similar ψ_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 ψ_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 these differences (21). Because exogenous nutrients 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 ψ_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 tested. In this study, sclerotial germination was not greatly reduced by CO_2 concentrations up to 9%, although linear mycelial growth on soil was affected. Griffin 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 1.2% 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 Griffin 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 Beute and Rodriguez-Kabana (3) reported that removal 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 sclerotial 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* predominantly 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 deeply 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 impinged on sclerotia by the surrounding soil. These pressures would vary with texture (sand:silt:clay ratio) and bulk density (grams per unit of volume) of the soil. With an average sclerotial diameter of about 2 mm, numerous points of contact between soil particles and the surface of the sclerotium should occur in most soils (except the very coarse sands), ensuring that the pressure is manifested uniformly over the sclerotial surface. Similar uniform atmospheric pressures (in the range of 0.05–0.1 atmospheres) achieved within a pressure bomb, however, did not prevent germination of sclerotia, indicating that pressure from physical contact with soil particles or a weight was different (Z. K. Punja and S. F. Jenkins, *unpublished*). When the vertical pressure imposed on sclerotia by a column of moistened soil was alleviated by a crack or by creating an air space above the sclerotia, germination was promoted. Similarly, when sclerotia were buried under a tightly packed column of vermiculite that imposed a pressure of <0.002 kg/cm^2, germination was not inhibited. Although Abegunawardena and Wood (1) reported that passage of air through a soil column improved germination of buried sclerotia, we did not observe such an effect. In fact, their data (1) indicated that germination at the soil surface also was enhanced, suggesting that some fungistatic factor(s) was annulled by aeration. Although ethylene was reported to inhibit sclerotial germination in soil at 1 μg/ml (31), results from this and another study (3) failed to demonstrate any reduction in germination by C_2H_4, even at 40 μg/ml. The marked inhibition by 1 μg/ml of C_2H_4 reported by Smith (31) may have been coincidental or may have affected germination indirectly.

Leakage of amino compounds and carbohydrates was increased by imposing pressure over sclerotia. Exudation of these or similar materials from sclerotia was reported to increase activities of soil microorganisms (8,14), which frequently resulted in rapid sclerotial decomposition (8,30). Numerous investigators have shown that sclerotia buried in soil survive for shorter periods than those at the soil surface (4,6,11,18). Increased leakage from sclerotia under pressure imposed by soil at greater depths could contribute to their reduced survival. In fact, sclerotia under weights or those buried in soil frequently were colonized more in our study than those at the surface or without simulated pressure. The manner in which leakage was increased is unknown; apparently, however, damage or cracking of the rind may not contribute significantly to reducing survival because this previously was shown to increase leakage only slightly (25). Drying and subsequent remoistening of sclerotia also has been reported to increase leakage (8,25,30) and dried sclerotia survived for shorter periods when buried than did nondried sclerotia (8). Survival was less in moist than in dry soil (4) and in the presence of volatile compounds or plant tissues (4,22). These studies indicated that when the activities of soil microorganisms near sclerotia were increased, survival was reduced. Although the sclerotia used in this study were dried before incubation in moist soil, thus increasing the likelihood for colonization (8,30), nondried sclerotia subjected to pressure from weights also leaked and were colonized. These observations indicate that deep burial of sclerotia in soil where they could conceivably be subjected to increased pressure and subsequent leakage would inhibit their germination and reduce their survival. In fact, deep burial has been recommended (and shown) to reduce disease caused by *S. rolfsii* (5,6,11,13,33). The reduction in disease was attributed by some investigators to the removal of organic matter from the soil surface,

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

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. Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. Agric. Exp. Stn. Tech. Bull. 174. 202 pp.
3. 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.
4. Beute, M. K., and Rodriguez-Kabana, R. 1981. Effects of soil moisture, temperature, and field environment on survival of *Sclerotium rolfsii* in Alabama and North Carolina. Phytopathology 71:1293-1296.
5. Boyle, L. W. 1961. The ecology of *Sclerotium rolfsii* with emphasis on the role of saprophytic media. Phytopathology 51:117-119.
6. Chowdhury, S. 1945. Diseases of pan (Piper betle) in Sylhet, Assam. V. Sclerotial wilt. Indian Acad. Sci. Proc. (Sect. B) 22:175-190.
7. Coley-Smith, J. R., and Cooke, R. C. 1971. Survival and germination of fungal sclerotia. Annu. Rev. Phytopathol. 9:65-92.
8. Coley-Smith, J. R., Ghaffar, A., and Javed, Z. U. R. 1974. The effect of dry conditions on subsequent leakage and rotting of fungal sclerotia. Soil Biol. Biochem. 6:307-312.
9. Dubey, H. D. 1958. Relation of soil texture and occurrence of root rot disease (*Sclerotium rolfsii* Sacc.) of peanut. Plant Dis. Rep. 42:1376-1377.
10. Duniway, J. M. 1979. Water relations of water molds. Annu. Rev. Phytopathol. 17:431-460.
11. Fajardo, T. G., and Mendoza, J. M. 1935. Studies on the *Sclerotium rolfsii* Sacc. attacking tomato, peanuts, and other plants in the Philippines. Philipp. J. Agric. 6:387-424.
12. Flados, N. D. 1958. Ecological factors affecting growth and formation of sclerotia in *Sclerotium rolfsii*. Ph.D. dissertation, Texas A&M College. 56 pp.
13. Garren, K. H. 1961. Control of *Sclerotium rolfsii* through cultural practices. Phytopathology 51:120-124.
14. Gilbert, R. G., and Linderman, R. G. 1971. Increased activity of soil microorganisms near sclerotia of *Sclerotium rolfsii* in soil. Can. J. Microbiol. 17:557-562.
15. Griffin, D. M., and Nair, N. G. 1968. Growth of *Sclerotium rolfsii* at different concentrations of oxygen and carbon dioxide. J. Exp. Bot. 19:812-816.
16. Grogan, R. G., Sall, M. A., and Punja, Z. K. 1980. Concepts for modeling root infection by soilborne fungi. Phytopathology 70:361-363.
17. Higgins, B. B. 1927. Physiology and parasitism of *Sclerotium rolfsii*. Phytopathology 17:417-448.
18. Javed, Z. U. R., and Coley-Smith, J. R. 1973. Studies on germination of sclerotia of *Sclerotium delphinii*. Trans. Br. Mycol. Soc. 60:441-451.
19. Kritzman, G., Chet, I., and Henis, Y. 1977. Effect of carbon dioxide on growth and carbohydrate metabolism in *Sclerotium rolfsii*. J. Gen. Microbiol. 100:167-175.
20. Leach, L. D., and Davey, A. E. 1938. Determining the sclerotial population of *Sclerotium rolfsii* by soil analysis and predicting losses of sugar beets on the basis of these analyses. J. Agric. Res. 56:619-631.
21. Lifshitz, R., and Hancock, J. G. 1983. Saprophytic development of *Pythium ultimum* in soil as a function of water matric potential and temperature. Phytopathology 73:257-261.
22. Linderman, R. G., and Gilbert, R. G. 1969. Stimulation of *Sclerotium rolfsii* in soil by volatile components of alfalfa hay. Phytopathology 59:1366-1372.
23. Mitchell, D. J., and Mitchell, J. E. 1973. Oxygen and carbon dioxide concentration effects on the growth and reproduction of *Aphanomyces euteiches* and certain other soil-borne plant pathogens. Phytopathology 63:1053-1059.
24. 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.
25. Punja, Z. K., and Grogan, R. G. 1981. Eruptive germination of sclerotia of *Sclerotium rolfsii*. Phytopathology 71:1092-1099.
26. 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.
27. Punja, Z. K., and Grogan, R. G. 1982. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology 72:635-639.
28. Ramarao, P., and Raja, U. 1980. Effect of soil moisture on development of foot and root rot of wheat and on other soil microflora. Indian J. Mycol. Plant Pathol. 10:17-22.
29. Robinson, R. A., and Stokes, R. H. 1955. Electrolyte Solutions. Academic Press, New York. 512 pp.
30. Smith, A. M. 1972. Nutrient leakage promotes biological control of dried sclerotia of *Sclerotium rolfsii* Sacc. Soil. Biol. Biochem. 4:125-129.
31. Smith, A. M. 1976. Ethylene in soil biology. Annu. Rev. Phytopathol. 14:53-73.
32. Tabak, H. H., and Cooke, W. B. 1968. The effects of gaseous environments on the growth and metabolism of fungi. Bot. Rev. 34:126-252.
33. Taubenhaus, J. J. 1919. Recent studies on *Sclerotium rolfsii* Sacc. J. Agric. Res. 18:127-138.
34. Weber, G. F. 1932. Diseases of peppers in Florida. Fla. Agric. Exp. Stn. Bull. 244. 46 pp.
35. Weerapat, P., and Schroeder, H. W. 1966. Effect of soil temperature on resistance of rice to seedling blight caused by *Sclerotium rolfsii*. Phytopathology 56:640-644.