Behavior of Sclerotia of Sclerotium rolfsii Produced in Soil or in Culture Regarding Germination Stimulation by Volatiles, Fungistasis, and Sodium Hypochlorite Treatment

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ABSTRACT

Sclerotia of *Sclerotium rolfsii* produced in soil or culture were both stimulated to germinate on soil when exposed to vapors of volatile growth stimulants. Treatment with sodium hypochlorite (NaOCl) enhanced germination of both types of sclerotia without volatile stimulants. Sclerotia produced in culture were more sensitive to NaOCl than sclerotia produced in soil. Sclerotia produced in culture did not germinate on soil after 10-min treatment with NaOCl and most were bleached after 15 min. In contrast, sclerotia produced in soil generally tolerated 30 min in NaOCl and were not bleached at the end of that time. Since these results suggest structural and/or physiological differences, the use of sclerotia produced in soil rather than in culture is advocated for soil ecological studies. The procedures for producing soil sclerotia are presented.

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Sclerotium rolfsii Sacc. survives in soil primarily by means of sclerotia which are readily produced on infected plants and on colonized residues in soil. Sclerotia are also readily produced in laboratory cultures. Ease of production in culture is probably the main reason sclerotia from cultures have been used most often in research on this pathogen. The possibility exists, however, that sclerotia grown in culture may be physiologically different from those produced under natural field conditions in soil. In addition, variability of research results may stem from differences in type of medium used, age of cultures, degree of washing sclerotia in removing them from culture, and subsequent time period and conditions of storage.

Since we were interested in studying sclerotia most like those produced in the field, we employed a method for producing quantities of sclerotia directly in soil. These soil sclerotia (SS) were relatively undisturbed by experimental manipulations and were compared with sclerotia produced in culture on an artificial medium (CS) in a study wherein germination and sensitivity to sodium hypochlorite were examined.

METHODS AND RESULTS.–*Production of sclerotia.*–An isolate of *S. rolfsii* from soybean (*Glycine max* [L.] Merr.) was used in this study. Sclerotia were produced in soil by inoculating a layer of plant tissue which was buried in soil in a container. Approximately 500 cc of field soil were placed in the bottom of a 15-cm plastic pot (subsoil) (Fig. 1), followed by 25-50 g fresh plant tissue (usually tomato leaves and stems) which was inoculated with chopped pieces of culture of *S. rolfsii* actively growing on potato-dextrose agar (PDA). The inoculated residue was covered with 500 cc of field soil (casing layer). Water was added to moisten the soil to at least field capacity. Any excess water was absorbed through the bottom of the pot by the paper towel beneath. Drying of the soil surface was reduced by placing a 15-cm petri dish lid on the interior ledge of the pot, but off center to allow some air exchange. The pots were incubated at approximately 25 C for 14 days. During this period the fungus colonized the plant tissue, and mycelium grew up through the casing layer of soil, spread over the surface, and produced a surface layer of sclerotia (Fig. 2-A). As the sclerotia matured (Fig. 2-B), the connecting hyphae lysed, leaving the sclerotia free in or on the soil. The surface sclerotia were readily removed for experimental use either individually with a needle or by suction into a vacuum flask. The remaining buried

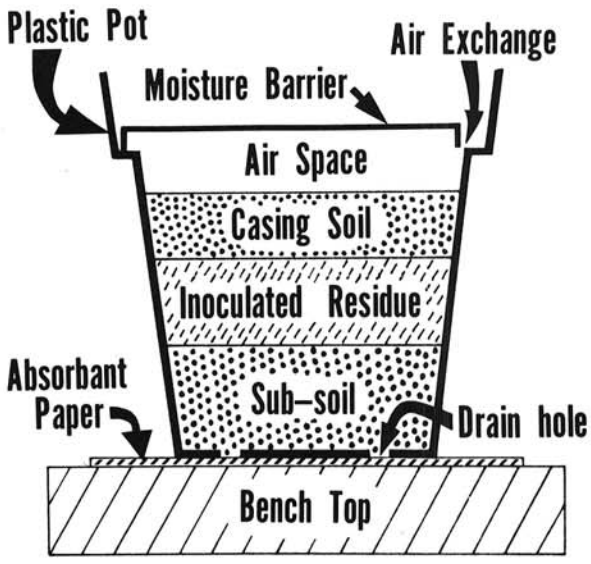

Fig. 1. Diagram of system used for producing sclerotia of *Sclerotium rolfsii* directly in soil.

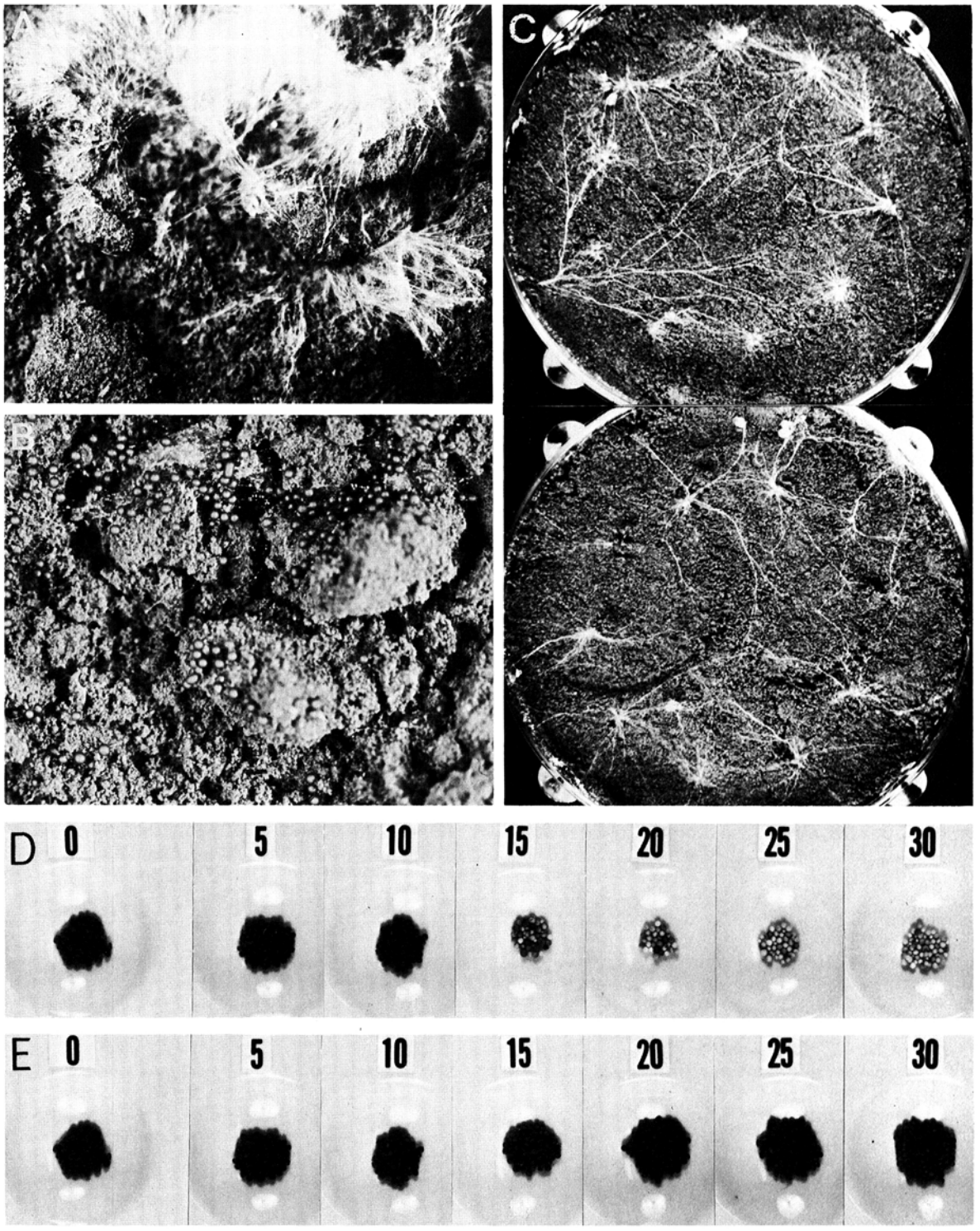

Fig. 2, A-E. A) Mycelium of *Sclerotium rolfsii* growing up through casing soil from inoculated tomato tissue. **B)** Mature sclerotia of *S. rolfsii* on the casing soil surface following lysis of connecting hyphae. **C)** Soil sclerotia of *S. rolfsii* which have germinated in response to alfalfa volatile stimulants (top) or as a result of a 20-min treatment in 0.5% sodium hypochlorite (NaOCl) without any volatile stimulants (bottom). **D, E)** Sclerotia of *S. rolfsii* treated for various time periods up to 30 min in NaOCl. Water controls (0) were treated for 15 min in water. **D)** Culture sclerotia showing nonuniform bleaching of the sclerotial rind after 15-30 min in NaOCl. **E)** Soil sclerotia showing no bleaching of sclerotial rind even after 30 min in NaOCl.

sclerotia could be removed by screening the soil and residue through 24-mesh soil screens, or they could be left in the soil as inoculum.

Tomato and soybean were the best of several plant species tried as substrates for sclerotial production in the soil. However, some plant materials supported little or no visible mycelial growth or sclerotial production. Notable among them was alfalfa hay, known to contain volatile compounds which stimulate sclerotial germination (9).

Sclerotia from cultures were produced on PDA slants. When they appeared mature (dark brown) after 3-4 weeks, they were aseptically removed from the cultures by tapping the tube to shake the sclerotia loose, and stored in the lab until used.

Germination of sclerotia.—Our earlier report (9) that volatile compounds from alfalfa hay stimulated germination of *S. rolfsii* sclerotia in soil was based on sclerotia (CS) produced on PDA. In this study we compared SS to CS with regard to their response to the volatile stimulants. The assay used was the same as previously described (9), wherein sclerotia were pressed into field soil in small (50 × 12 mm) plastic snap-lid petri dishes. Drops of alfalfa distillate (AD) were placed on the lid so that stimulatory vapors passed across the air gap to the soil and sclerotia. Vigor of mycelial growth from sclerotia was rated after 5 days. Both SS and CS were similarly stimulated by the AD vapors. In control soil dishes exposed only to water vapors, however, CS nearly always produced one or two germ tubes, in contrast to SS which usually produced none. Since SS were produced in soil, their reduced germination could have been due to inhibitory organisms on their surfaces. Therefore, we soaked them for various lengths of time up to 30 min in 0.5% sodium hypochlorite (NaOCl) to determine whether surface disinfestation would enhance their germination. CS produced under aseptic conditions were used in parallel treatments. Control SS and CS were soaked for 15 min in water. All sclerotia were air-dried for 1 hr following treatment and were then exposed to vapors from AD or water in the germination assay.

The results of these tests (Fig. 2-C, 3) indicated that all SS treated with NaOCl germinated in soil better than nontreated sclerotia in the absence of the AD stimulant. CS treated with NaOCl for 5-9 min germinated better than control sclerotia in the absence of the AD stimulant. Treatment of CS for 10 min or longer appeared to be lethal in that germination was markedly reduced. The slight enhancement of germination vigor of NaOCl-treated sclerotia in the presence of stimulatory AD vapors was apparent after 5-7 min for CS and between 10 and 20 min for SS. Since the CS were not contaminated prior to treatment, the enhanced germination cannot be accounted for by the removal of inhibitory organisms, but must involve some alteration of the physiology or structure of the sclerotia. Further evidence of the differences between these sclerotial types was the differential sensitivity to the NaOCl treatment with regard to mortality end point and to bleaching of the sclerotial rind. Many CS

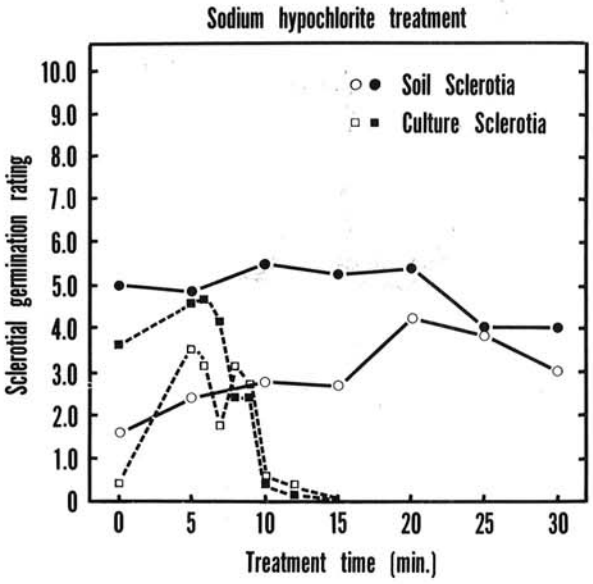

Fig. 3. Effects of 0.5% sodium hypochlorite treatment on germination of soil (circle data points) vs. culture (square data points) sclerotia of *Sclerotium rolfsii*. After treatments for various times, sclerotia were exposed to stimulatory volatiles from alfalfa (solid circles or squares) or water vapors (open circles or squares). Water controls were washed 15 min in distilled water (indicated as 0 on the treatment time scale).

were apparently killed by 10 min in NaOCl (as indicated by reduced germination ratings), whereas most of the SS tolerated at least 30 min in NaOCl (Fig. 3). Many CS were bleached by 10-15 min in NaOCl, whereas none of the SS were bleached, even after 30 min in NaOCl (Fig. 2-D, E).

DISCUSSION.—We have compared sclerotia of *S. rolfsii* produced in soil or in culture with respect to their germination response to volatile stimulants and their response to treatment with NaOCl. Whereas both types of sclerotia were stimulated to germinate by volatiles, their differential response to NaOCl suggests differences in rind thickness, composition, or permeability. In addition, sclerotia produced in culture were generally less uniform both in appearance and in responses than those produced in soil. These structural and/or physiological differences could well have direct implications on their relative capacities to germinate or survive in soil. Even though NaOCl enhanced germination of both sclerotial types in the absence of volatile stimulants, sclerotia produced in culture were more sensitive to NaOCl than soil sclerotia, perhaps because they have a thinner rind. The hypothesis could be extended that NaOCl increased the exudation of both sclerotial types. The work of Coley-Smith & Dickinson (2), which showed that surface sterilization of sclerotia of *S. cepivorum* Berk. with CaOCl enhanced exudation of bacteria-stimulating substances, supports this hypothesis. The further work of Coley-Smith and associates (3, 4, 6) with *S. cepivorum* also supports

the theory that sclerotia do not germinate in nonsterile soil because of the inhibitory substances being produced by soil bacteria at the soil-sclerotium interface. But since surface sterilization with CaOCl increased sclerotial exudation of bacteria-stimulating substances (2), one would expect treated sclerotia to be even more fungistatic. According to Allen & Young (1), surface sterilization of *S. cepivorum* sclerotia, which had been removed from soil, with NaOCl enhanced sclerotial germination in sterile water but not on nonsterile soil. They concluded that germination was enhanced in sterile water because inhibitory organisms on the sclerotial surface had been removed by the surface sterilization, whereas putting them back in soil re-established the surface flora of inhibitory organisms. They did not, however, state the duration of treatment or the concentration of the NaOCl solution. We must then reconcile the NaOCl-enhanced germination response of *S. rolfsii* sclerotia in light of the above reports on *S. cepivorum* and current thinking on fungistasis in general (7, 11). We might have interpreted our germination response with *S. rolfsii* as Allen & Young (1) did with *S. cepivorum*, except that NaOCl-enhanced germination also occurred with uncontaminated culture sclerotia. We therefore should consider two possibilities: (i) that NaOCl treatment nullifies an internal dormancy in the sclerotia, which contributes to their fungistasis; or (ii) that NaOCl treatment enhanced sclerotial exudation which resulted in microbial activity that stimulated rather than inhibited sclerotial germination, or both (i) and (ii). In support of the second possibility (ii) is our recent work (5) wherein we demonstrated that sclerotial exudate was responsible for the rapid increase of bacteria adjacent to *S. rolfsii* sclerotia in soil (mycosphere effect), and that these bacteria had a greater in vitro effect on *S. rolfsii* than nonmycosphere bacteria. Mycosphere bacteria were shown to be generally more stimulatory or inhibitory (depending on the medium) in paired culture with *S. rolfsii* than nonmycosphere bacteria. We further demonstrated that exposure of soil to sclerotia or concentrated sclerotial leachate, especially in the presence of alfalfa volatiles, increased the fungistatic potential of that soil to sclerotial germination (10). Thus, we have described a microenvironment at the soil-sclerotium interface in which mycosphere organisms, which have been selectively stimulated by sclerotia, may have both an inhibitory as well as a stimulatory effect on sclerotia. The only apparent differential factor is the increase (or possible qualitative change) in the amount of exudation (of nutrients) from the NaOCl-treated sclerotia. Those additional nutrients could create a nutrient-rich environment in which specific mycosphere organisms could produce and release metabolites which stimulate germination. Prior treatment of soil with alfalfa volatiles could enhance microbial activity enough to create a nutrient-deficient environment (7) in which mycosphere organisms respond by producing inhibitors, or by not supplying the stimulators needed for germination. The alfalfa volatiles themselves can stimulate sclerotial germination on nontreated soil (9), but we have evidence which suggests that the volatiles, at least in part, stimulate the mycosphere organisms which in turn stimulate sclerotial germination (Linderman & Gilbert, *unpublished data*).

Watson & Ford (11) neglected to cite our work on *S. rolfsii* which bears specifically on the subject of stimulation and inhibition of sclerotial germination in soil in relation to fungistasis (5, 8, 9, 10), but we believe this work supports their conclusions that soil fungistasis is controlled by a complex balance of stimulators and inhibitors in soil microenvironments, and that both exogenous and endogenous factors, acting concurrently, consecutively, or both, may be involved in causing fungistasis. We also agree with the suggestion (11) that further evidence to support our hypothesis should be obtained from qualitative and quantitative chemical analysis of specific metabolites exchanged between a fungus propagule and its associated organisms, such as the sclerotial exudates following NaOCl treatment.

In view of the comparative similarities and differences which we have reported for sclerotia produced in soil or in pure culture, we advocate that soil sclerotia should be used for ecological studies of *S. rolfsii* in soil. They are easy to produce by the method described, and they appear to be structurally and physiologically more uniform than those produced in culture. Their use would contribute to a more meaningful and uniform literature on the ecology of *S. rolfsii* in soil.

LITERATURE CITED

1. ALLEN, J. D., & J. M. YOUNG. 1968. Soil fungistasis and Sclerotium cepivorum Berk. Plant Soil 29:479-480.
2. COLEY-SMITH, J. R., & D. J. DICKINSON. 1971. Effects of sclerotia of Sclerotium cepivorum Berk. on soil bacteria. The nature of substances exuded by sclerotia. Soil Biol. Biochem. 3:27-32.
3. COLEY-SMITH, J. R., J. E. KING, D. J. DICKINSON, & R. W. HOLT. 1967. Germination of sclerotia of Sclerotium cepivorum Berk. under aseptic conditions. Ann. Appl. Biol. 60:109-115.
4. DICKINSON, D. J., & J. R. COLEY-SMITH. 1970. Stimulation of soil bacteria by sclerotia of Sclerotium cepivorum Berk. in relation to fungistasis. Soil Biol. Biochem. 2:157-162.
5. GILBERT, R. G., & R. G. LINDERMAN. 1971. Increased activity of soil microorganisms near sclerotia of Sclerotium rolfsii in soil. Can. J. Microbiol. 17:557-562.
6. KING, J. E., & J. R. COLEY-SMITH. 1969. Suppression of sclerotial germination in Sclerotium cepivorum Berk. by water expressed from four soils. Soil Biol. Biochem. 1:83-87.
7. KO, W. H., & J. L. LOCKWOOD. 1967. Soil fungistasis: Relation to fungal spore nutrition. Phytopathology 57:894-901.
8. LINDERMAN, R. G., & R. G. GILBERT. 1968. Stimulation of Sclerotium rolfsii and its antagonists by volatile components of alfalfa hay. Phytopathology 58:1057 (Abstr.).

9. LINDERMAN, R. G., & R. G. GILBERT. 1969. Stimulation of Sclerotium rolfsii in soil by volatile components of alfalfa hay. Phytopathology 59:1366-1372.

10. LINDERMAN, R. G., & R. G. GILBERT. 1972. Influence of volatile compounds from alfalfa hay on microbial activity in soil in relation to growth of Sclerotium rolfsii. Phytopathology 63:359-362.

11. WATSON, A. G., & E. J. FORD. 1972. Soil fungistasis – a reappraisal. Annu. Rev. Phytopathol. 10:327-348.