

# Stimulation of *Sclerotium rolfsii* in Soil by Volatile Components of Alfalfa Hay

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## ABSTRACT

Sclerotia of *Sclerotium rolfsii*, placed on or buried in moist field soil, were stimulated to germinate and grow on or through the soil by vapors of volatile compounds in a distillate of alfalfa hay, or by vapors from alfalfa hay residue. Vapor concentrations above the level that gave maximum stimulation were inhibitory or lethal. Sclerotia were stimulated to germinate by distillate vapors that passed through at least 5 cm of a soil column, but mycelial growth to the soil surface occurred only when the soil depth was 2 cm or less.

The volatile components of alfalfa appear to serve primarily as stimulants, and not as growth substrates or nutrients. Exposures as short as 15 min stimulated as much mycelial growth as exposures for 5 days. Linear mycelial growth on nutrient agar was not stimulated at lower vapor concentrations, and was inhibited during the first 24 hr at the higher

vapor concentrations. Although the stimulatory capacity of the drops of distillate was effective during 15-min exposures, such capacity was not reduced after 1 hr, owing to dissipation or uptake by sclerotia and soil.

The compounds identified in the distillate (i.e., methanol, acetaldehyde, isobutyraldehyde, and isovaleraldehyde) in a reconstituted mixture stimulated *S. rolfsii* nearly as much as did the crude alfalfa hay distillate. To a lesser degree, these compounds singly, except isobutyraldehyde, stimulated *S. rolfsii*.

Survival of sclerotia in soil was reduced by exposure to vapors of the alfalfa distillate, owing to expenditure of the sclerotial energy source, lysis of resulting mycelium, and increased colonization of sclerotia by soil antagonists and saprophytes. Phytopathology 59:1366-1372.

Plant residues in soil may play very important roles in the etiology of root diseases. Residue decomposition products may stimulate germination of pathogen propagules that increases the incidence of disease, or may predispose roots to increased infection. To a "necrotrophic pathogen" such as *Sclerotium rolfsii* Sacc., the presence of plant debris in the soil is thought to be essential for establishment of a parasitic relationship with its host (3, 4, 8, 14). Boyle has suggested (4) that the sclerotium of *S. rolfsii* does not contain enough energy to parasitize the host without first colonizing some organic matter, thus building up sufficient energy to produce metabolites necessary to initiate pathogenesis.

Ecologically, *S. rolfsii* survives in soil by means of resistant sclerotia which, under appropriate conditions, germinate to give rise to the vegetative mycelium or pathogenic phase (4). Just what factor(s) triggers the transition from the resting sclerotium to vegetative mycelial growth in soil is not clear. It is known, however, that the disease caused by *S. rolfsii* is favored by good soil aeration, high soil moisture, and high temperatures. Yet even when all these factors are favorable, no disease occurs unless undecomposed plant residue is present (4, 6). Boyle (2) has proposed capitalizing on this fact in control practices by ". . . denying the organism a medium for growth in the upper stratum of soil, where conditions are most favorable for its development". It is not certain, however, that eliminating organic residue from the soil *only* denies *S. rolfsii* a growth medium for development prior to parasitism. The plant residues may also provide the necessary chemical stimulus for mycelium to grow from the sclerotium to the residue or the host. We investigated the efficacy of volatile compounds from alfalfa hay (9, 16, 17) to stimulate sclerotial germination and growth in soil, and the results follow. A preliminary report has been published (13).

**MATERIALS AND METHODS.**—Sclerotia of two clones of *Sclerotium rolfsii* Sacc. isolated from soybean (*Glycine max* [L.] Merr.) (SR-1) and white clover (*Trifolium repens* L.) (SR-2) were produced on potato-dextrose agar (PDA) slants, and removed when mature. They were dried at room temperature (23 C) and stored until used. Germination tests on PDA showed that nearly all were viable, some even after 3 years.

To test the behavior of the sclerotia in soil, we placed sclerotia on 3 g of nonsterile Warden soil in sealable, snap-lid petri dishes. Warden soil is a sandy clay loam with a pH of 7.3 to 7.5, organic matter content of 0.8%, cation exchange capacity of 120 meq/100 g, and a water-holding capacity of 12%. The soil was kept frozen or refrigerated until used to ensure relative uniformity between experiments. Soil was pressed into the dishes and moistened slightly by atomizing water over the surface. This procedure established a uniform soil moisture level and structure. Ten sclerotia were spaced evenly in each dish and pressed into the soil. Treatment of the soils and the sclerotia was accomplished by hanging drops (one drop was approximately 0.037 ml) of alfalfa distillate, water, or solutions of the known components of the alfalfa distillate from the dish lid before sealing. This allowed only vapors to serve as stimulants. Germination counts and estimations of mycelial growth from sclerotia were made 4-6 days after treatment. Ratings of 0 for no germination to 10 for germination and maximum mycelial growth were used.

The alfalfa (*Medicago sativa* L.) hay distillate used in these studies was prepared as described by Menzies & Gilbert (16). For comparative purposes, dosages were adjusted by varying the volume, i.e., number of drops, of crude distillate to which soil dishes with sclerotia were exposed. The major components of this distillate active as soil respiration stimulants have recently been identi-

fied (17) as methanol, acetaldehyde, isobutyraldehyde, and isovaleraldehyde. The concentrations of these substances in the crude alfalfa distillate were determined by gas chromatographic analysis to be: methanol, 1.03%; acetaldehyde, 0.24%; isobutyraldehyde, 0.12%; and isovaleraldehyde, 0.26%. Synthetic solutions of these compounds, prepared singly and in a mixture at their crude distillate concentrations, were used in some experiments.

In some experiments, dry sclerotia were added to fresh Warden soil in polyethylene bottles, and held at room temperatures for from several days to several weeks before being used. The sclerotia were recovered from this soil by screening through a series of soil sieves, the smallest of which was mesh 24. These sclerotia were then removed from the sieves, pressed into the soil in test dishes, and exposed to distillate vapors.

The response of sclerotia to alfalfa distillate or chopped alfalfa hay, when the sclerotia were covered with or buried in soil, was also determined. Sclerotia were either placed on the bottom of snap-lid dishes and covered with 3 g of soil, or placed in soil in 18-mm sealed test tubes and covered with varied depths of soil. The stimulatory vapors came from 0.5 ml of alfalfa distillate or 0.5 g air-dried, chopped, moistened alfalfa hay placed in a shell vial on the soil surface.

Survival of sclerotia which were buried in soil (50 sclerotia/10 g soil) in sealed vials and exposed either to water or 0.5 ml or 1.0 ml of alfalfa distillate was determined. Three weeks after burial, the sclerotia were screened from the soil and tested for germinability on moistened filter paper.

To determine the influence of the volatile compounds on mycelial growth in vitro on PDA plates or 2% purified agar (Difco), we inoculated agar snap-lid dishes with uniform inoculum plugs. The initial colony perimeter was marked, and the colonies were exposed to water, or to 1, 2, 4, 8, 12, or 16 drops of alfalfa distillate. Colony diameters were then measured after 24, 48, and 72 hr.

**RESULTS.—Stimulation of sclerotial germination and growth.**—When dry sclerotia of *S. rolfsii* clones SR-1 and SR-2 were placed in moistened Warden soil and exposed to vapors of alfalfa distillate, germination was stimulated. Mycelial growth over the soil surface was evident after 36-48 hr, as compared to water controls in which few sclerotia germinated. Those developed only sparse mycelium. A dosage response curve was established for this stimulation response with clone SR-1. The dosage increased as the number of drops of distillate placed on the lid of the test dish increased (Fig. 1, 2). The response was greatest when eight drops of the distillate were used. Sclerotial germination and mycelial growth were almost completely inhibited when treatments of 27 drops (approximately 1.0 ml)/dish were used. Those that survived, however, grew rapidly and profusely when the distillate vapor source was removed.

Germination and mycelial growth of sclerotia placed singly on 2% purified agar were stimulated by the distillate vapors, except at the higher dosages where mycelial growth was inhibited. Removal of the distillate vapor source at those dosages allowed vigorous mycelial

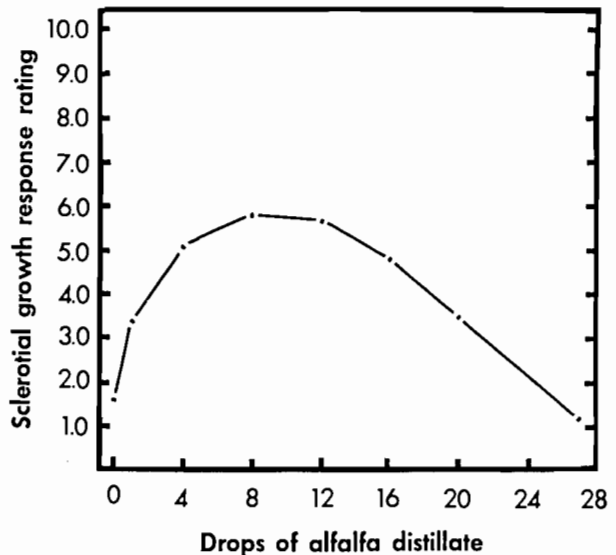


Fig. 1. Sclerotial growth response ratings indicating the degree of stimulation of sclerotial germination and growth of *Sclerotium rolfsii* on soil exposed to vapors of alfalfa distillate. The alfalfa distillate vapor concentration was increased by increasing the number of drops hanging from the dish lid (1 drop was approximately 0.037 ml).

growth. Very little mycelial growth occurred in the water controls.

The in vitro inhibition of mycelial growth by distillate vapors was further examined by exposing mycelium growing on PDA or 2% purified agar plates without sclerotia to vapors of the alfalfa distillate or water. These studies (Fig. 3-A, 4-A) showed that linear mycelial growth on PDA was initially (24 hr) inhibited by higher vapor concentrations, but that inhibition was overcome in 48 to 72 hr. Mycelial growth was much denser in PDA plates exposed to alfalfa distillate as compared to water controls. Mycelial growth on PDA was predominantly oriented to the distillate drops, and sclerotial formation, although delayed, was more predominant in or around the drops. Linear mycelial growth on purified water agar was not increased by the distillate vapors.

We tested the possibility that sclerotia, buried in soil, might not respond in the same manner as those placed on the soil surface. Sclerotia were buried in soil in a closed container and held at room temperature for 1 month. They were then recovered by soil screening, transferred to soil dishes, and exposed to water or alfalfa distillate vapors. Sclerotia exposed to water vapors did not germinate, whereas those exposed to vapors from 4, 8, or 12 drops of alfalfa distillate germinated, and the mycelial growth was extensive.

The vapors from the alfalfa distillate also stimulated sclerotia that were covered by soil. Sclerotia were placed in the bottom of the test dishes, covered with 3 g of soil, and exposed to vapors of alfalfa distillate. They were stimulated to germinate, and the mycelium grew up through the soil toward the hanging drops (Fig. 3-C).

To determine the depth of soil that the alfalfa distillate vapors would penetrate and stimulate sclerotia

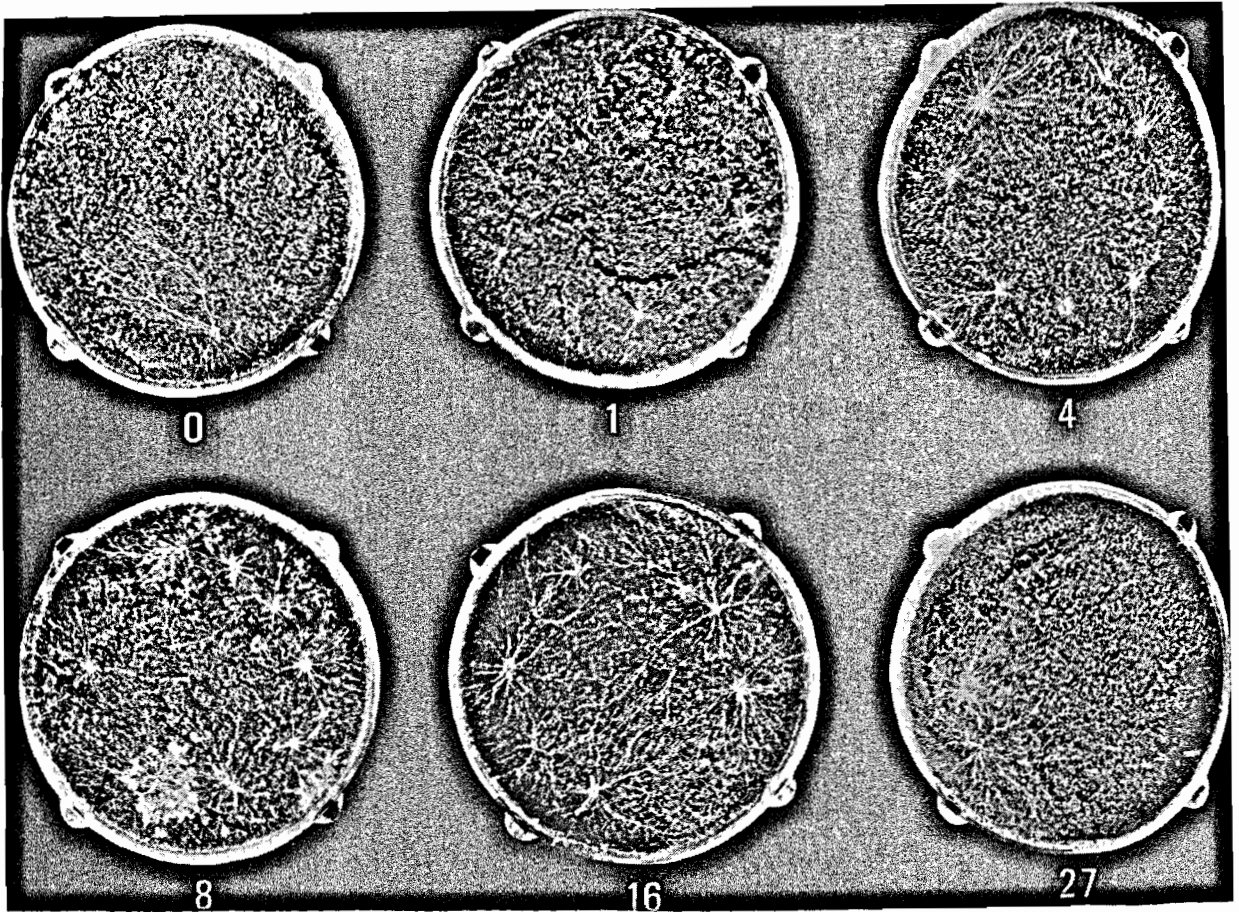


Fig. 2. Sclerotial growth response of *Sclerotium rolfsii* to vapors from an increasing number of drops of alfalfa distillate.

to germinate, we varied the depth of soil that covered sclerotia. A constant volume (0.5 ml) of the distillate in a vial on the soil surface served as the stimulus. Whether the sclerotia were stimulated to germinate and grow, and whether the mycelium grew to the soil surface, was recorded (Table 1). These observations showed that sclerotia, buried to a depth of 5 cm, were stimulated to germinate; but only those covered with 2 cm or less of soil produced growth that reached the soil surface in 1 week (Fig. 3-B).

TABLE 1. Stimulatory effect of vapors from alfalfa distillate on germination of sclerotia of *Sclerotium rolfsii* and growth of mycelium through various depths of soil

| Soil depth<br>cm | Germination |                |    |                 | Growth to soil surface |    |    |    |
|------------------|-------------|----------------|----|-----------------|------------------------|----|----|----|
|                  | 1a          | 1b             | 2a | 2b <sup>a</sup> | 1a                     | 1b | 2a | 2b |
| 0.6              | +           | +              | +  | +               | +                      | +  | +  | +  |
| 1.2              | +           | +              | +  | +               | +                      | +  | +  | +  |
| 1.9              | +           | +              | +  | +               | +                      | —  | —  | +  |
| 2.5              | +           | ± <sup>b</sup> | ±  | +               | —                      | —  | —  | —  |
| 3.1              | +           | ±              | +  | —               | —                      | —  | —  | —  |
| 3.8              | ±           | +              | —  | +               | —                      | —  | —  | —  |
| 4.4              | +           | +              | —  | —               | —                      | —  | —  | —  |
| 5.0              | +           | +              | +  | +               | —                      | —  | —  | —  |

<sup>a</sup> Experiment conducted twice, each time with two replications, a and b.  
<sup>b</sup> Weak, nonuniform germination.

Experiments were conducted to determine if the alfalfa hay itself could stimulate sclerotial germination and growth. Sclerotia were placed in or on soil and separated from chopped, moistened alfalfa hay by a soil layer. In some trials, the stimulus source was chopped, moistened alfalfa hay contained in a vial on the soil surface inside a test tube. In others, the snap-lid soil dishes were used. Regardless of method, volatile compounds emanating from the chopped alfalfa residue stimulated sclerotial germination and growth of *S. rolfsii* sclerotia.

*Influence of vapor exposure time on stimulation of S. rolfsii.*—The vapor exposure time needed to stimulate maximum sclerotial germination and growth was determined. Distillate hanging drops were removed from test dishes after various time intervals ranging from 2 min to 32 hr. We rated sclerotial growth response after 5 days, and compared them with ratings obtained from sclerotia continually exposed to the distillate for 5 days. Exposure of the sclerotia to the distillate for only 2 min gave some stimulation; exposure for 15 min was sufficient to trigger as much mycelial growth as exposure for 32 hr (Fig. 4-B, C). Inhibition of mycelial growth by 20 or 16 drops of distillate was evident after 1 and 8 hr, respectively; and the inhibition increased with longer exposure times.

To determine the time necessary to remove or reduce the sclerotial germination stimulants from the source

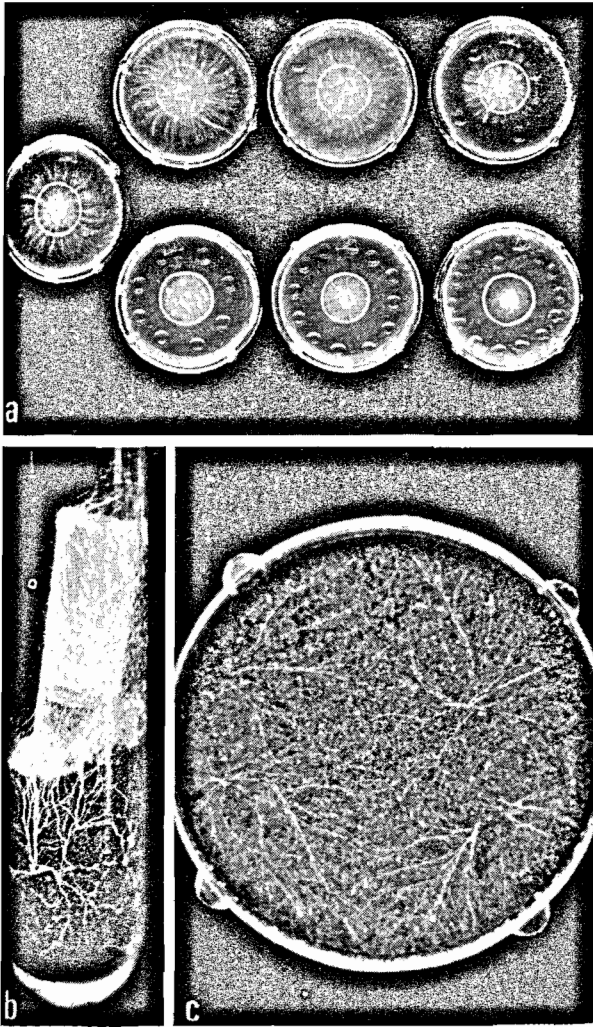
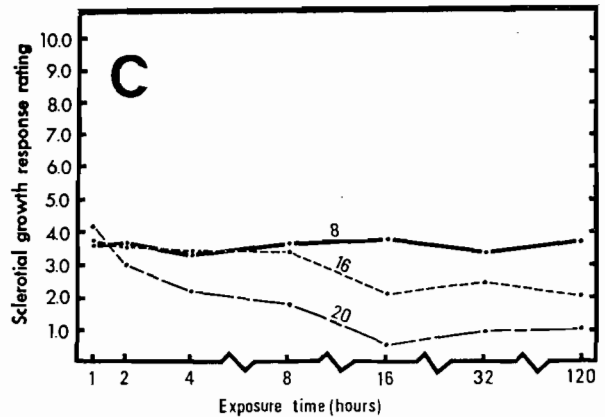
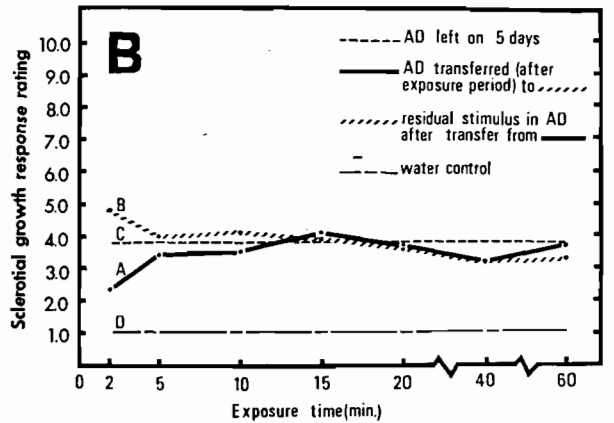
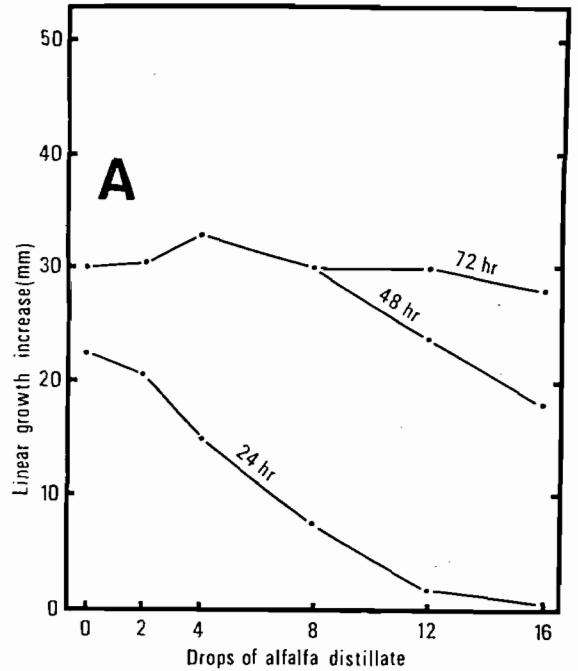


Fig. 3. a) Mycelial growth inhibition of *Sclerotium rolfsii* on PDA after 24-hr exposure to various vapor concentrations of alfalfa distillate: left to right (above): 1, 2, and 4 drops of distillate, (below): 8, 12, and 16 drops of distillate. Plate centered on the left is the water control. b) Mycelial growth resulting from stimulation of sclerotia, buried 2 cm deep in a soil column, by vapors from alfalfa distillate emanating from the vial on the soil surface. c) Mycelial growth from sclerotia covered with 3 g of soil and stimulated by vapors from hanging drops of alfalfa distillate.

drops (by sclerotial and soil uptake or by vapor dissipation due to leakage), we transferred the lid and distillate drops from one soil dish to a second soil dish (with sclerotia) after exposures of 2, 5, 10, 15, 20, 40, and 60 min. The sclerotial-stimulating capacity of the distillate drops was not reduced even in the 60-min exposure (Fig. 4-B). Serial transfers of distillate drops

Fig. 4. A) Linear growth increases of mycelium of *Sclerotium rolfsii* grown on PDA plates exposed to water or increased vapor concentrations of alfalfa distillate for 24, 48, and 72 hr. Each point was derived by subtracting the initial colony diameter from each time interval colony diameter measurement. B) Sclerotial growth response ratings of *Sclerotium rolfsii* 5 days after initial short-time-interval exposures to vapors of alfalfa distillate. After initial vapor exposure for each of the exposure times (A), distil-



late drops were transferred to new sclerotial soil dishes (B) where they remained for the duration of the 5-day experiment. Line (C) represents the growth response ratings for an 8-drop exposure which was left on the whole 5 days. Line (D) is the water control. C) Sclerotial growth response ratings of *Sclerotium rolfsii* 5 days after initial exposures of from 1-32 hr. Alfalfa distillate vapor concentrations released from 8, 16, or 20 drops were used. Note inhibition occurring from treatments of 20 and 16 drops after 1 and 8 hr, respectively.



to new sclerotial dishes, after 15-min exposures, showed that the sclerotial-stimulating capacity of the drops of distillate was not reduced even after ten 15-min transfers.

*Stimulation of S. rolfsii with known components of the alfalfa hay distillate.*—The response of sclerotia to four or eight drops of a synthetic mixture of the active (17) components and to the individual components was compared to that resulting from exposure to the crude distillate at equivalent concentrations. The growth ratings made after 4-6 days (Table 2) indicate that the mixture nearly duplicates the crude distillate in stimulating sclerotial germination and growth. However, the individual compounds were less stimulatory than the mixture. Isobutyraldehyde gave no stimulation of *S. rolfsii*.

*Survival of sclerotia in soil exposed to vapors of alfalfa distillate.*—We determined the fate of sclerotia in soil exposed to vapors of alfalfa distillate or water by direct observation of soil dishes, and by determining viability of sclerotia buried in soil in vials and exposed to water or the distillate vapors. Sclerotia exposed only to water vapors generally germinated with a sparse mycelium on the soil surface; but the sclerotium remained intact over several weeks of observation (Fig. 5-A). On the other hand, sclerotia stimulated to germinate and grow by the alfalfa distillate collapsed because of depletion or lysis of the inner sclerotial contents (Fig. 5-B). Unless a new secondary sclerotium formed (Fig. 5-C), that propagule of the fungus was lost from the soil. When placed on moist soil, some sclerotia were colonized by soil saprophytes or antagonists. These sclerotia, weakened either initially (mechanical) or by high vapor concentrations of alfalfa distillate, were more readily colonized when exposed to alfalfa distillate vapors than to water. Among those fungi most frequently observed colonizing sclerotia were *Chaetomium* (Fig. 5-D), *Trichoderma*, and *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans.

To determine if sclerotial numbers declined faster in distillate-exposed soils than in water-exposed soils, we mixed 50 sclerotia in 10 g of soil. An inner vial containing water, 0.5 ml, or 1.0 ml of alfalfa distillate was placed on the soil surface. After the vials had been sealed for 3 weeks, the sclerotia were recovered by screening. The total number of viable sclerotia recovered from two replications/treatment was determined by

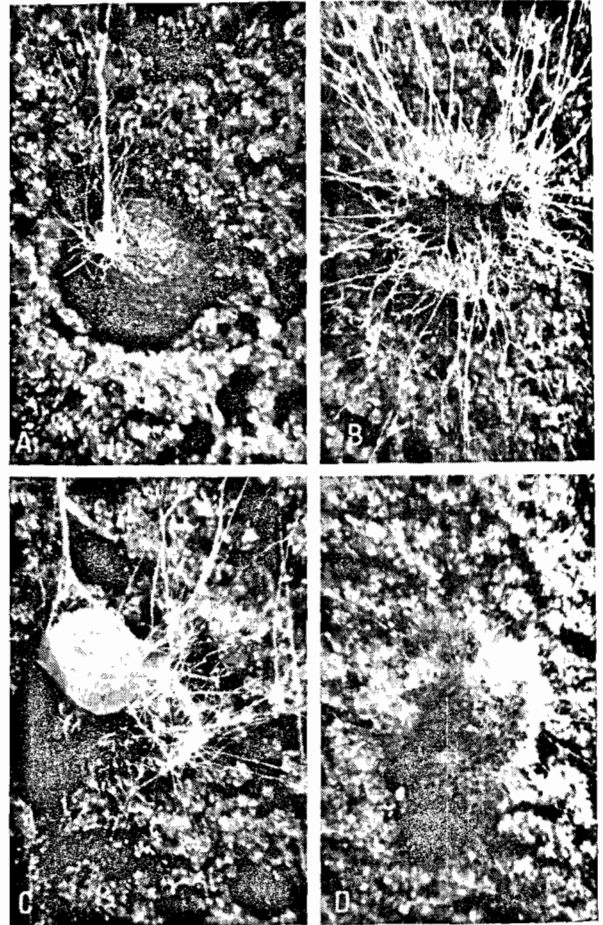


Fig. 5. Sclerotia of *Sclerotium rolfsii* on Warden soil exposed to water or vapors of alfalfa distillate. A) Sclerotium exposed to water showing retention of sclerotial integrity and viability. B) Sclerotium stimulated to germinate and grow by vapors of alfalfa distillate and ultimate collapse due to depletion of food reserves or microbial lysis. C) Sclerotium stimulated by vapors of alfalfa distillate that is forming a secondary sclerotium. D) Sclerotium colonized by a soil-inhabiting species of *Chaetomium* that has produced its perithecia on the sclerotium surface.

germinating them on moistened filter paper. This study showed that exposure to alfalfa distillate decreases the sclerotial population in soil more than does exposure to water (Table 3).

**DISCUSSION.**—*Sclerotium rolfsii* Sacc. is a soilborne pathogen of many woody and herbaceous host plants in many areas of the world (1). Observations that the disease caused by this fungus usually occurs only in the presence of undecomposed plant residue, even though other environmental conditions are favorable, have prompted workers (4, 8) to hypothesize that *S. rolfsii* must grow on an organic substrate before it can attack and parasitize a host. We investigated the possibility that plant debris may be necessary for reasons other than as a food base prior to infection. If a physical separation exists in the soil between the sclerotia of *S. rolfsii* and the host plant or undecomposed residue, some chemical stimulus is necessary to trigger sclerotial germination and mycelial growth toward the host or plant debris. As shown here, vapors of volatile

TABLE 2. Growth response ratings of sclerotia of *Sclerotium rolfsii* on soil to volatile components of alfalfa hay

| Treatment                | Growth response <sup>a</sup> |                  |
|--------------------------|------------------------------|------------------|
| H <sub>2</sub> O         | 0.8 <sup>b</sup>             | 0.9 <sup>c</sup> |
| Crude distillate         | 3.8                          | 5.3              |
| Mixture                  | 2.9                          | 4.5              |
| Methanol (1.08%)         | 1.6                          | 1.7              |
| Acetaldehyde (0.24%)     | 1.1                          | 1.7              |
| Isobutyraldehyde (0.12%) | 0.6                          | 1.0              |
| Isovaleraldehyde (0.26%) | 1.4                          | 2.0              |

<sup>a</sup> Avg. of 3 exp.; ratings: 0 = no germination, 10 = maximum germination and mycelial growth.

<sup>b</sup> Treatment of four drops/dish.

<sup>c</sup> Treatment of eight drops/dish.

TABLE 3. Survival of sclerotia of *Sclerotium rolfsii* in soil exposed for 3 weeks to alfalfa distillate (AD) or water

| Treatment        | Sclerotia recovered <sup>a</sup> |   | Germination <sup>b</sup> |
|------------------|----------------------------------|---|--------------------------|
|                  | No.                              | % |                          |
| H <sub>2</sub> O | 81                               |   | 83                       |
| 0.5 ml AD        | 69                               |   | 57                       |
| 1.0 ml AD        | 77                               |   | 43                       |

<sup>a</sup> Avg. of 3 exp. with 100 sclerotia/treatment buried in soil, 50/replication.

<sup>b</sup> Germination percentages based on No. of sclerotia recovered.

compounds that originate from alfalfa hay can stimulate sclerotial germination and mycelial growth in soil. Menzies & Gilbert (16) showed that volatiles which stimulated soil respiration occurred in corn leaves, bluegrass leaves, barley straw, and tea leaves, as well as in alfalfa hay. Owens et al. (17) demonstrated that alfalfa hay and green alfalfa differed quantitatively, but not qualitatively, as sources of the soil respiration stimulants. We have shown that these compounds, probably released by various plant residues, can stimulate sclerotial germination and growth of *S. rolfsii* in soil under experimental conditions. Moreover, these same compounds are apparently responsible for microbial responses by other soil fungi, such as the population increases and subsequent decline of *Verticillium dahliae* in soil reported by Gilbert et al. (10). Whether roots or other parts of hosts susceptible to *S. rolfsii* might also emit vapors of volatile compounds has not been determined. King & Coley-Smith (12), however, recently demonstrated stimulation of sclerotial germination of *S. cepivorum* by volatile components of host species of *Allium*. Dalal et al. (7) reported that ripening tomato fruit released volatile compounds which accounted for their odor. Among those compounds identified (primarily alcohols, aldehydes, and esters) were compounds which also occur in the alfalfa distillates: namely, ethanol, acetaldehyde, and isovaleraldehyde. Since *S. rolfsii* is known to cause a severe tomato fruit rot (6), one could speculate that these volatile compounds from the fruit may activate the sclerotia of the pathogen. In other cases, plant debris colonization by *S. rolfsii* may occur before infection merely because the residue (and not the host plant roots) produced the volatile stimulants necessary to stimulate sclerotial germination and mycelial growth through the soil to the residue. Since *S. rolfsii* can readily utilize residues as a food base, colonization of that residue occurs. As a result, the residue would have (i) activated *S. rolfsii*, and (ii) increased its potential as inoculum by bridging the gap between the sclerotium and host, and by providing an intermediate energy source. The question remains as to whether mycelium growing through soil could have parasitized a host plant prior to residue colonization. Our studies show that sclerotia of *S. rolfsii* contain adequate energy to grow considerable distances, through, and on soil if they receive an adequate stimulus.

Germination of sclerotia of *S. rolfsii* in soil is usually prevented by the overriding forces of fungistasis. It is

generally accepted that fungistasis is caused by microbial activity in the soil (15). It has been suggested that the nature of this fungistasis might be due to the production by soil organisms of some substance(s) in the soil that inhibits soil fungus propagule germination, or to a competition between the fungus propagule and other organisms for nutrients essential for that fungus propagule to germinate. The first theory has been challenged largely because of the lack of evidence for the existence in the soil of a "fungistatic factor". The nutritional theory has gained support from the fact that addition of nutrients often overcomes fungistasis. Theoretically, one could test the nutrition hypothesis by somehow stimulating fungus propagule germination without adding any external nutrients to the soil. This apparently has not been done, because stimulants that could be added in such brief exposures and then removed have not been reported. We feel that volatile compounds such as those present in the alfalfa distillate have tremendous potential in this regard. If short exposures to such ephemeral volatile compounds provide the stimulus necessary for sclerotial germination in *S. rolfsii* without adding any nutrient substrate to the system, then competition for available nutrients in soil is most likely not the limiting factor in its soil fungistasis. The vapors from the volatile compounds seem only to trigger mycelial growth that does not even appear until 36 hr after exposure. In vitro experiments, in addition, demonstrate that the volatiles do not increase the linear mycelial growth rate without the sclerotium. Further, the sparse mycelium that results from exposure to distillate vapors on purified agar did not produce any sclerotia, indicating the lack of available nutrients. Therefore, the extent of the mycelial growth coming from a stimulated sclerotium must be based on the food reserve stored in that sclerotium and activated or converted to usable energy by the stimulus. If the stimulus concentration is high (but not inhibitory), then a maximum amount of mycelial growth results; if it is low, less mycelium grows. The concentration of stimulus that actually reaches the sclerotia determines whether they will germinate at all, and how far the mycelium grows through the soil. Once germination occurs, the extent of growth at any level of stimulus may be limited by the level of antagonism in the soil.

Menzies & Gilbert (16) and Gilbert et al. (9) showed a striking increase in microbial activity in soil exposed to vapors of alfalfa hay distillate. We have reported here that the volatile compounds may influence *S. rolfsii* directly by stimulating sclerotial germination. But other organisms are also stimulated, and this results in an increased colonization of weakened sclerotia (5) and a more rapid lysis of mycelium. These indirect effects may prevent formation of secondary sclerotia, and thus decrease the sclerotial inoculum of the soil. Exposure of soil to volatile compounds may increase the antagonistic potential of that soil and thereby establish a biological control of *S. rolfsii*. Henis & Chet (11) have already emphasized the potential of such an approach when they demonstrated that nitrogenous amendments caused an increase in the microflora antagonistic to *S. rolfsii*.

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