

## Factors Affecting Germinability and Susceptibility to Attack of Sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum* in Field Soil

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We thank Ahuva Henis and Kimberly Christensen for skillful technical assistance. We also thank M. Dunn for helping in the identification of the indigenous soil microorganisms isolated from the sclerotia.

Accepted for publication 4 May 1983.

### ABSTRACT

Henis, Y., and Papavizas, G. C. 1983. Factors affecting germinability and susceptibility to attack of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum* in field soil. *Phytopathology* 73:1469-1474.

Fresh, soil-produced, and PDA-produced sclerotia of *Sclerotium rolfsii* were exposed to drying, washing, NaClO (1% solution), metham-sodium, and heat shock treatments. The treated sclerotia were immersed in spore suspensions of *Trichoderma harzianum* (WT-6) and incubated on field soil and on potato-dextrose agar (PDA) supplemented with 10 µg of 8-hydroxyquinoline (PDA-8HQ) per milliliter. Sclerotia were examined for ability to germinate on PDA-8HQ, the ability of WT-6 to sporulate on their surface, and for their eventual degradation. Naturally or artificially produced fresh sclerotia neither germinated nor were they attacked by WT-6 in field soil. Exposure of sclerotia to a relative humidity of ≤30% (≤7.12 mm Hg) for 24–48 hr, to ≥100 µg of metham-sodium per gram of soil for 1–4 hr, and to localized heat treatment of 90 C for 15 sec, triggered

their germination in field soil and increased their susceptibility to degradation by WT-6. Germination of the pretreated sclerotia in soil was mainly of the eruptive type except following NaClO treatment. An additive effect of metham-sodium pretreatment and WT-6 inoculation was demonstrated with 80–1,000 µg of fumigant per gram of soil for 1–4 hr or with 20 µg/g for 16 hr in field soil. Treatment of sclerotia with metham-sodium before inoculation with *T. harzianum* (WT-6) resulted in an increase in sporulation of WT-6 on the sclerotia and an increase of susceptibility of sclerotia to degradation by the antagonist. This treatment also resulted in a lag phase longer than that of untreated sclerotia germinated on an agar medium.

It is well known that sclerotia of *Sclerotium rolfsii* Sacc. survive a long time and have high resistance to microbial attack in soil. Fresh sclerotia of *S. rolfsii* incubated in soil did not germinate, but remained viable during exposure to the indigenous soil microbiota (16,17) or to added *Trichoderma harzianum* Rifai (7) even following penetration (6). According to Smith (17,18), dried sclerotia rotted within 2–3 wk in moist soil because they leaked nutrients which provided sufficient colonization potential to allow the soil microbiota to overcome sclerotial defenses. Smith (17) also noticed that drying the sclerotia, or alternately washing and drying

them until all soluble nutrients had been removed, stimulated their germination in soil. Gilbert and Linderman (4) demonstrated increased activity of soil microorganisms near dried sclerotia of *S. rolfsii*. They did not, however, examine the behavior of fresh sclerotia. Beute and Rodriguez-Kabana (1) demonstrated a stimulatory effect of remoistened dried peanut stems and leaves on sclerotial germination. Linderman and Gilbert (11) reported that sclerotia of *S. rolfsii* produced in soil or culture were stimulated to germinate by vapors of volatiles from alfalfa distillate and by sodium hypochlorite (NaClO) treatment. Punja and Grogan (14,15) conditioned sclerotia of *S. rolfsii* to germinate eruptively in soil by drying them for 7–10 hr at 15–20% relative humidity (RH) or exposing them to volatile substances from plant tissues. This was also accomplished, but to a lesser extent, by washing them with running water, treating them with NaClO, or puncturing the sclerotia.

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Although considerable research has been done on germination of sclerotia of *S. rolfssii*, no attempts have been made to relate the effect of various treatments on both germination and degradation in soil. The purpose of this work was to examine the effect of physical and chemical factors on germination of sclerotia of *S. rolfssii* in soil and on their susceptibility to attack by both the indigenous soil microbiota and by an isolate of the antagonistic fungus, *T. harzianum*.

## MATERIALS AND METHODS

Strain Sr-3 of *S. rolfssii*, provided by R. Rodriguez-Kabana, Auburn University, Auburn, AL 36830, and strain WT-6 of *T. harzianum* provided by H. D. Wells, Tifton, GA 31794, were used throughout. Strain WT-6 was easily distinguishable from other strains or species of *Trichoderma* because it produced white conidial masses, which was not characteristic of any of the indigenous *Trichoderma* spp. in the soil. Conidia of *T. harzianum* were produced by growing the isolate on Difco potato-dextrose agar (PDA) for 7–8 days under continuous fluorescent light ( $\sim 700 \mu\text{Ein}/\text{m}^2/\text{sec}$ ). Conidia were removed from the agar surface by pipetting 3–5 ml of sterile distilled water on the surface and gently rubbing the surface with a sterile cotton-tipped applicator. Conidia were counted in a hemacytometer, and suspensions were adjusted to provide the desired density of conidia in each test.

Sclerotia of *S. rolfssii* were produced on PDA in petri plates or on field soil supplemented with oat kernels (5). Germinability of sclerotia was assayed on PDA supplemented with 8-hydroxyquinoline at 10  $\mu\text{g}/\text{ml}$ , which selectively inhibited germination of conidia of *T. harzianum*, but not that of the sclerotia of *S. rolfssii* (5).

**Soil plates for sclerotial germination.** Sassafras loamy sand

TABLE 1. Effect of *Trichoderma harzianum* strain WT-6 on fresh and dried sclerotia of *Sclerotium rolfssii* strain Sr-3<sup>a</sup> produced on PDA

Treatment of sclerotia	Sclerotial germination (%)	Sclerotial <sup>b</sup> degradation (%)	New sclerotia produced/plate (no.)
Dried	100	0	10 a <sup>c</sup>
Dried plus WT-6	0	100	2 bc
Fresh	100	0	3.3 b
Fresh plus WT-6	0	0	0 c

<sup>a</sup>Fresh and dried sclerotia were immersed in aqueous suspensions of conidia of WT-6 ( $1.5 \times 10^7/\text{ml}$ ) for 30 min and incubated on water agar for 3 wk at 28 C.

<sup>b</sup>Sclerotia collapse upon touching.

<sup>c</sup>Values followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test.

TABLE 2. Effect of drying and washing of naturally produced sclerotia of *Sclerotium rolfssii* strain Sr-3 on their germinability and susceptibility to degradation by *Trichoderma harzianum* strain WT-6<sup>a</sup>

Treatment	Sclerotial germination on soil (%)	Sclerotial degradation (%) <sup>b</sup>
<i>No Trichoderma</i>		
Fresh	0 a <sup>c</sup>	0 a
Fresh-washed	0 a	0 a
Dried	90 d	30 b
Dried-washed	71 c	8 a
<i>Trichoderma added</i>		
Fresh	0 a	0 a
Fresh-washed	0 a	0 a
Dried	6 ab	84 c
Dried-washed	15 b	88 c

<sup>a</sup>Dried and washed sclerotia were immersed in aqueous suspensions of conidia of WT-6 ( $1.5 \times 10^7/\text{ml}$ ) for 30 min and incubated on natural soil for 7 days at 28 C.

<sup>b</sup>Sclerotia collapse upon touching.

<sup>c</sup>In each column, values followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test.

(SLS) (pH 6.2, 1.5% organic matter) was sieved through a 0.85-mm sieve and its moisture content was adjusted to about –2 bars (approximately 80% of moisture holding capacity). The soil was distributed in petri plates (60 g per plate, wet weight) and the surfaces were smoothed with a glass cover from a 50-mm-diameter petri plate. Nontreated and pretreated 3-wk-old sclerotia were placed on the soil surface (20 per plate) and pushed into the soil with a glass rod so that only their tops were exposed. The plates were sealed in polyethylene bags and incubated at 28 C for 7 days. Each treatment was replicated five times.

**Pretreatment of sclerotia.** Sclerotia of strain Sr-3 were exposed to the following pretreatments in order to study their effect on germination and susceptibility to attack (degradation and sporulation) by *Trichoderma*: (i) Sclerotia were dried by placing them in a desiccator over calcium sulfate at 20 C. Relative humidity was monitored with a Brannan Mini-Combination Thermometer-Hygrometer (E. C. Geiger, Harleysville, PA 19438). The sclerotia were kept in the desiccator until the humidity dropped to 10% (vapor pressure = 2.4 mm Hg). (ii) Fresh and dried sclerotia were washed for 2 hr with tap water running at the rate of 1 L/min. (iii) Sclerotia were pretreated with a 1% solution of NaClO for 5 min and washed on a filter with 2 L of sterile distilled water. (iv) Sclerotia were pretreated with metham-sodium (sodium *N*-methylthiocarbamate; Vapam®, Stauffer Chemical Co., Dayton, NJ 08810) either by immersion for 2 hr in an aqueous solution of metham-sodium (1,000  $\mu\text{g}$  of metham-sodium per milliliter), or by injecting a 0.6-ml aliquot of an aqueous solution of the chemical into a sealed glass vial (18  $\times$  65 mm) containing a mixture of 100 sclerotia and 12 g SLS at a water potential of –0.7 bars. After exposure for 2, 4, or 18 hr, sclerotia were separated from the soil in the vials by sieving and washed with running tap water to remove soil particles. These sclerotia, and those from the aqueous metham-sodium solution, were washed with sterile distilled water as in (ii). (v) Sclerotia were heat-treated by placing several sclerotia to the center of an agar plate within a circle 5–6 cm in diameter, filling a 100-ml Pyrex beaker (with a flat bottom) with boiling water, and pressing the sclerotia into the agar with the beaker bottom for 15 sec. The recorded water temperature during the 15-sec period was 90 C. Sclerotia exposed to treatments (i)–(v) were either used as such or were inoculated with *T. harzianum* by immersion in aqueous suspension of conidia of WT-6 ( $1.5 \times 10^7/\text{ml}$ ) for 30 min at room temperature. Treated and nontreated sclerotia were placed on SLS plates (20 per plate) and the plates were incubated at 28 C for 7 days.

**Definition of terms.** Sclerotia were considered degraded when they lost firmness and collapsed upon application of slight pressure with a forceps. The word "attack" is used synonymously with "degradation and sporulation" by *Trichoderma*.

## RESULTS

**Effect of drying of sclerotia on germinability and susceptibility to attack by *T. harzianum*.** Fresh and dried sclerotia produced on PDA were immersed in a spore suspension of *T. harzianum* (WT-6) and incubated on water agar (WA, 2% agar, 10 sclerotia per plate) and on SLS (20 sclerotia per plate) at 28 C for 7 days. The presence of WT-6 inhibited germination of both fresh and dried sclerotia on WA, but it degraded only dried sclerotia on this substrate (Table 1). When sclerotia were plated on field soil, strain WT-6 sporulated well after 3 days on dried, but not on fresh sclerotia. The dried sclerotia were degraded after 7 days, but the fresh ones were not. Fresh, unwashed PDA-produced sclerotia did not germinate when incubated on field soil for 7 days at 28 C, but dried, unwashed sclerotia germinated eruptively. Germination of dried, washed sclerotia was similar to that of dried unwashed sclerotia. When WT-6 was added to dried, washed sclerotia, no germination occurred and all sclerotia were visibly colonized by the antagonist.

In another test, sclerotia produced on natural soil were incubated on SLS (Table 2). Fresh, unwashed sclerotia did not germinate and were not attacked by WT-6 or by the indigenous microbiota. In contrast, dried, unwashed sclerotia germinated eruptively and 84% were degraded when WT-6 was present and 30% in its absence.

Washing the dried sclerotia for 2 hr did not change the germinability and degradation patterns. Similar results were obtained with sclerotia produced on PDA.

**Effect of NaClO and metham-sodium.** Sclerotia produced on PDA were immersed in 1% aqueous solution of NaClO for 5 min or in an aqueous solution of metham-sodium (1,000 µg/ml) for 2 hr, washed with distilled water, and incubated on SLS for 7 days with or without WT-6. Both chemical treatments induced sclerotial germination (Table 3). Metham-sodium induced germination of the eruptive type (14,15) and made the sclerotia vulnerable to WT-6. Sodium hypochlorite only induced noneruptive germination (by single hyphae) without affecting sclerotial susceptibility to WT-6.

**Effect of preincubation of sclerotia in soil containing metham-sodium.** Samples of 100 sclerotia produced on PDA were mixed with 12 g SLS (at -0.7) and the mixture was placed in glass vials (6.5 × 18 mm diameter). Aqueous metham-sodium solutions were injected (0.6 ml per vial) into the sealed soil-sclerotia mixture in the vials with a hypodermic syringe to give final concentrations equivalent to 0, 20, 40, 80, 100, and 1,000 µg metham-sodium per gram of soil. One set of five vials was opened after 1, 2, 4, and 18 hr. The sclerotia were collected by wet sieving, washed with running distilled water, and divided into two batches. One batch was not treated further. The second batch was immersed in a conidial suspension of WT-6 (1.5 × 10<sup>7</sup> conidia per milliliter). Sclerotia from both batches were placed on SLS and on PDA-8HQ plates, and all were incubated at 28 C for 7 days.

Fresh, uninoculated, PDA-produced sclerotia that had been exposed to 1,000 µg of metham-sodium per gram of soil for 4 or 18 hr, and those exposed to 100, 80, and 40 µg per gram of soil for 18 hr, did not germinate in soil (Fig. 1). Sclerotia exposed to 1,000 or 100 µg of metham-sodium per gram of soil for 1 or 2 hr germinated (eruptively) 25 and 35% and 10 and 20%, respectively. Sclerotia that had been exposed to metham-sodium before being inoculated with conidia of WT-6 were colonized and degraded by WT-6. The extent of degradation depended on the chemical concentration and length of exposure to the biocide. For example, with the highest

metham-sodium concentration, and after 1 hr of exposure, WT-6 resulted in 10% germination whereas metham-sodium alone allowed 35% germination. Fresh sclerotia did not germinate on SLS, but 60% of the sclerotia that had been incubated in soil vials without metham-sodium for 18 hr germinated on the soil plates.

The relationship between susceptibility of the metham-sodium-pretreated sclerotia to WT-6 on soil plates and their inherent viability as evaluated on PDA-8HQ was also examined. Seventy-seven percent of the sclerotia exposed for 1 hr to 1,000 µg of metham-sodium per gram of soil germinated on PDA-8HQ; on the other hand, 80% of the sclerotial population exposed to the biocide at 1,000 µg/g and treated with WT-6 showed degradation by and sporulation of WT-6 (Fig. 2). Similar relationships can be seen with all metham-sodium concentrations tested (including 20 µg/g) depending on concentration and time of exposure. Water controls for all treatments had 98–100% viability.

TABLE 3. Effect of chemical treatments<sup>w</sup> on germinability and susceptibility of sclerotia of *Sclerotium rolfsii* strain Sr-3 to attack and degradation by *Trichoderma harzianum* strain WT-6<sup>x</sup>

Treatment	Sclerotial germination (%)	Sclerotial degradation (%) <sup>y</sup>
<i>No Trichoderma</i>		
Fresh	0 a <sup>z</sup>	0 a
Fresh-NaClO	76 b	0 a
Fresh-metham-sodium	99 c	0 a
<i>Trichoderma added</i>		
Fresh	0 a	0 a
Fresh-NaClO	91 c	6 a
Fresh-metham-sodium	0 a	98 b

<sup>w</sup>Sclerotia produced on PDA were immersed in 1% NaClO for 5 min or in aqueous solution of metham-sodium (1,000 µg/ml) for 2 hr.

<sup>x</sup>Sclerotia were immersed in aqueous suspensions of conidia of WT-6 (1.5 × 10<sup>7</sup>/ml) for 30 min and incubated on natural SLS soil for 7 days at 28 C.

<sup>y</sup>Sclerotia collapse upon touching.

<sup>z</sup>In each column, values followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's multiple range test.

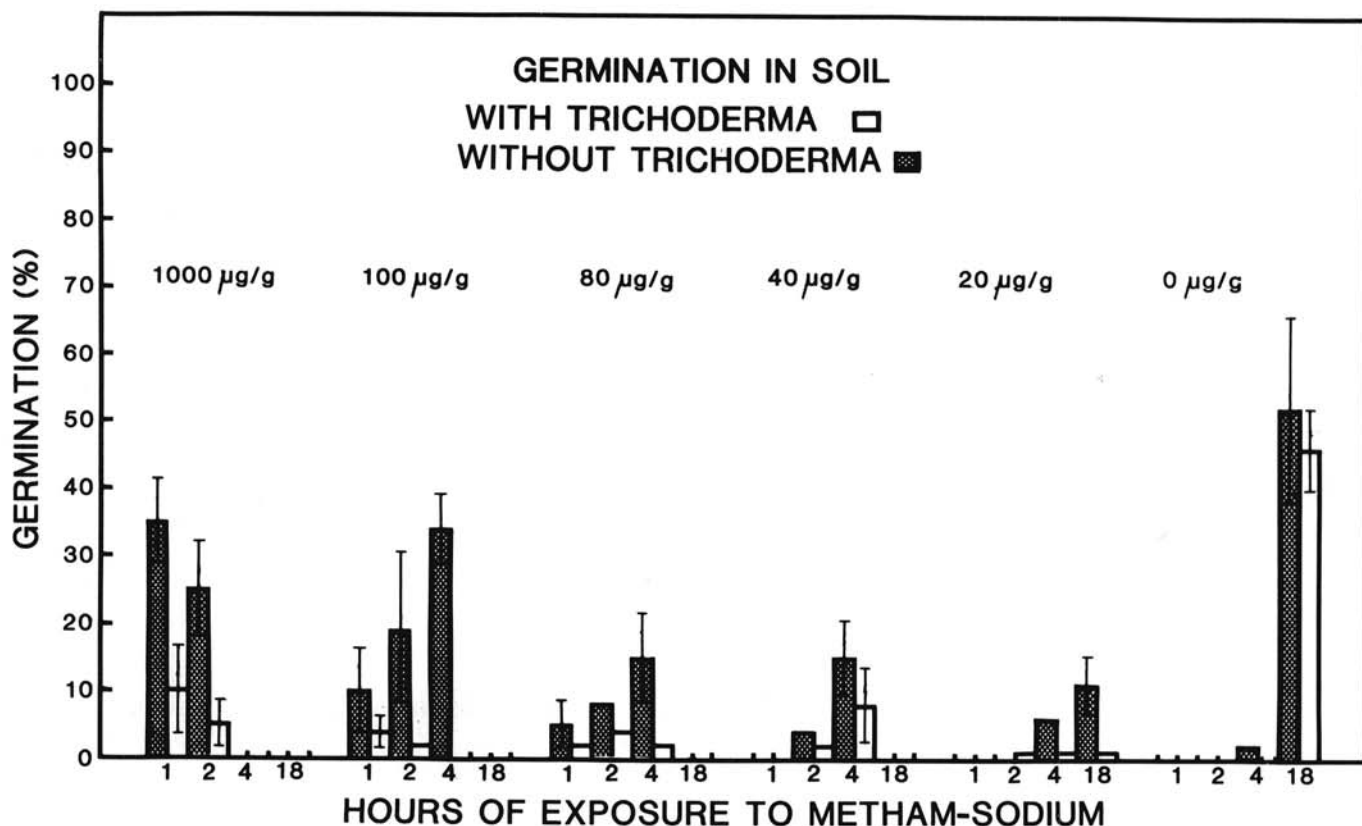


Fig. 1. Effect of metham-sodium pretreatment in soil on germinability of potato-dextrose agar-produced sclerotia of *Sclerotium rolfsii* strain Sr-3 on soil after 7 days of incubation.

Pretreatment with metham-sodium at all concentrations tested also increased the numbers of sclerotia degraded by indigenous fungal flora in the absence of WT-6 inoculation (Fig. 3). The extent of colonization increased with increasing concentrations of metham-sodium and increased length of exposure. Most common among the fungi isolated from the pretreated sclerotia were: *Fusarium* spp., *Gliocladium virens*, *T. hamatum*, *T. harzianum*, and *Mucor* spp.

The effect of metham-sodium pretreatment on viability of sclerotia in soil was tested by using a high concentration (1,000  $\mu\text{g/g}$ ) with short exposure and low concentration (100  $\mu\text{g/g}$ ) with long exposure. Sclerotia from such treatments were recovered from soil, washed, and plated on PDA-8HQ to assay germinability. Sclerotia exposed to 1,000  $\mu\text{g/g}$  of metham-sodium in soil for 4 hr did not germinate on PDA-8HQ even after 7 days of incubation (Fig. 4). The germination of those exposed to the low concentration for 4 hr began after a 4-day lag period and after 7 days reached 70%. Sclerotia exposed to the high and low concentration of the fumigant for 2 hr had a lag phase of 2 and 1 days, respectively, and ultimately germinated about 15 and 60%, respectively. No lag phase was observed and germination exceeded 75% after 1 hr of exposure to both concentrations. Sclerotia exposed to soil with no metham-sodium germinated almost 100%.

**Effect of partial heat treatment and mechanical damage.** Heat treatment of sclerotia placed on agar with the bottom of a flat Pyrex beaker containing water at 90 C for 15 sec caused some injury to the cells of the outer sclerotial rind tissue, while the lower part, being in the agar, remained protected. The treated sclerotia germinated almost 100%. Other sclerotia were punctured with a

pin, a mechanical damage previously reported to enhance germinability of sclerotia on WA (2). Sclerotia exposed to these treatments were either used as such or were inoculated with WT-6. Eighty-five percent of the heated sclerotia were degraded by WT-6. Nonheated sclerotia and punctured sclerotia were not degraded by WT-6. When the heat-treated area of the sclerotia was marked with a liquid-ink marker before transfer to soil, germination of the partially heated sclerotia took place at or near the treated area.

## DISCUSSION

Fresh sclerotia of *S. rolfii* produced on PDA or on natural soil neither germinated nor were degraded by *T. harzianum* (WT-6) in field soil (Table 1). Lack of germination in nonsterile soil in the absence of a susceptible host could be attributed to soil fungistasis (11,13) and sclerotial resistance to microbial attack could be accounted for by the presence of melanin in their rind (2). The effect of drying on sclerotial behavior in soil was first noticed by Smith (16) who showed that dried sclerotia of *S. rolfii* germinated and were more readily attacked by the indigenous soil microbiota than were fresh ones. Our results corroborate Smith's findings with respect to the effects of drying.

Eruptive germination of sclerotia of *S. rolfii* followed by production of new sclerotia was first reported by Punja and Grogan (14,15). Our results showed for the first time that sclerotia of *S. rolfii* can be triggered to germinate eruptively not only by drying, but also by treatment with metham-sodium and by partial heat treatment. These treatments resulted in an increased susceptibility of sclerotia to attack and degradation by *T. harzianum*. It could be

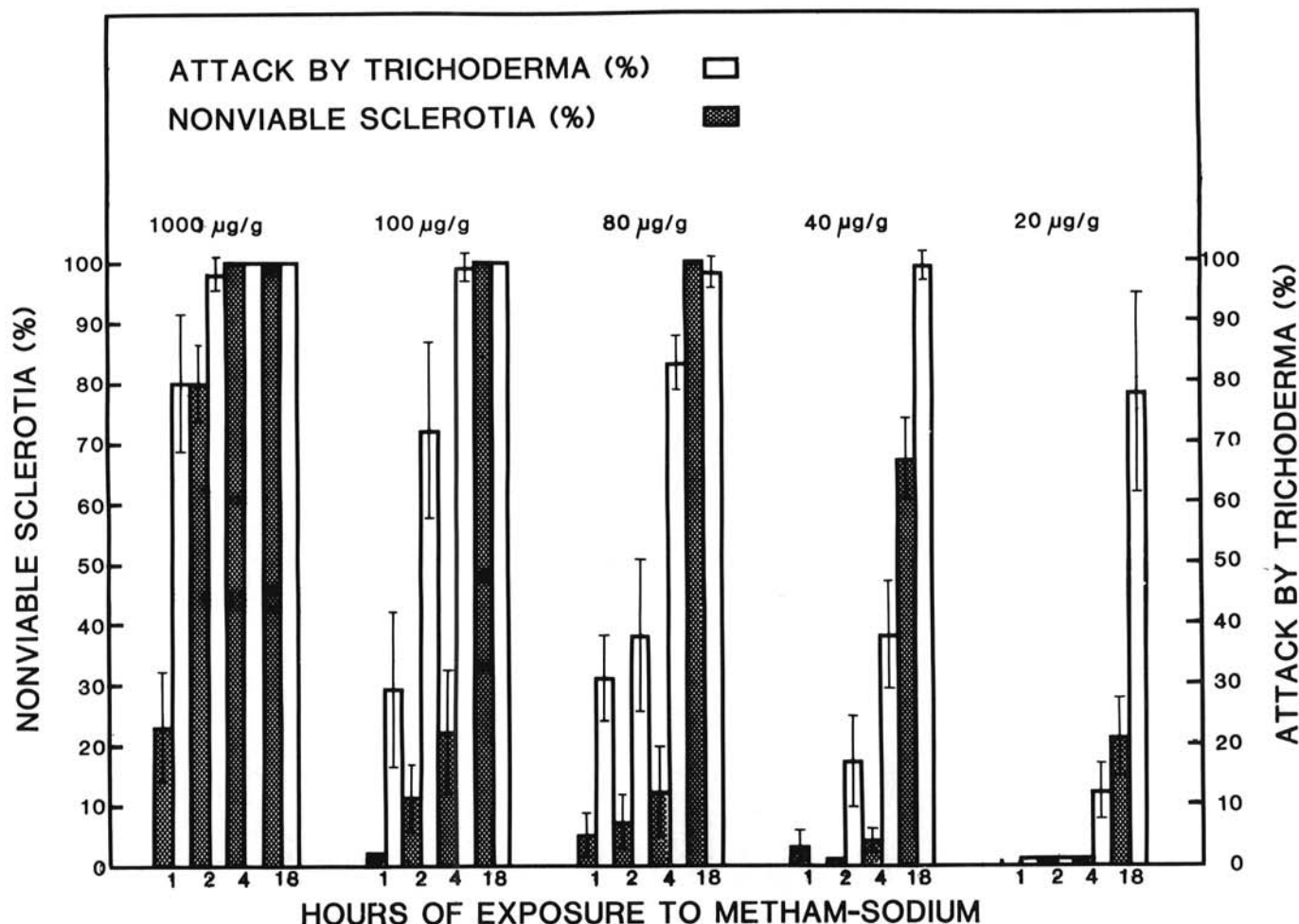


Fig. 2. Effect of metham-sodium pretreatment on viability and susceptibility of sclerotia of *Sclerotium rolfii* strain Sr-3 to degradation by *Trichoderma harzianum* (WT-6). Following pretreatment, sclerotia were placed on potato-dextrose agar-8 hydroxyquinoline medium and incubated for 4 days to determine viability. Other samples were immersed in the antagonist suspension ( $1.5 \times 10^7$  conidia per milliliter) and incubated in soil for 7 days. Water controls run for all treatments had 98–100% viability and 0–2% attack by *T. harzianum*.

argued that germinating sclerotia became susceptible to the antagonist as a result of the germination process. This suggestion, however, is not supported by experimental evidence since sclerotia triggered to germinate by overnight incubation in moist soil remained resistant to attack by *T. harzianum*. Furthermore, every inoculated sclerotium was immersed in the same WT-6 spore suspension and yet the few of those inoculated sclerotia that managed to germinate (even eruptively) were attacked by *T. harzianum*. The possibility that *T. harzianum* (WT-6) parasitized the mycelium of *S. rolfssii* that had emerged from the sclerotium is not supported by the experimental evidence. The antagonist was unable to parasitize young, living, vegetative mycelium of *S. rolfssii*. These observations corroborate those of Smith (16) who also reported lack of relationship between sclerotia germination and microbial degradation in soil. These considerations, however, do not exclude later microbial degradation of the remnants of the germinating sclerotium as well as lysis of mycelia of *S. rolfssii* by the soil microflora.

It has been shown (3,17) that germinating sclerotia of *S. rolfssii* excrete amino acids and sugars. Increased excretion of such substances by damaged sclerotia could be involved in both germination of sclerotia and attack by antagonists as shown by Smith for dried sclerotia (16-18). However, the washing of dried sclerotia for 2 hr prevented neither attack and degradation by WT-6 nor eruptive sclerotial germination. These results do not agree with the finding of Smith (17) who reported that overnight immersion of dried sclerotia in water reduced sclerotial degradation by the soil microbiota without affecting germinability. These differences in results might stem, among other factors, from the slow rate of nutrient leakage of the sclerotial cells. Alternatively, the chemical or physical shock applied to the sclerotia could have triggered a continuous nutrient release that still operated during and after washing. In this case, the pathogen

and its mycoparasite might have competed for nutrients inside the sclerotium. Thus, the use of this newly available food base by either the pathogen or the antagonist could result in a repression of either the antagonist or germination by the sclerotia of the pathogen.

The extent that damage to the sclerotial rind is involved in the predisposition of dried sclerotia to attack by *Trichoderma* is not known. In contrast to drying, sclerotia were not predisposed to attack by mechanical damage (puncturing). This finding does not support the contention that the condition of the sclerotial rind is the only factor to explain the predisposition mechanism.

Slow release of nutrients may be important to both *S. rolfssii* and *T. harzianum* in overcoming soil fungistasis. Under soil fungistasis in field soil the reserve nutrients of the sclerotium may remain unavailable to the sclerotial cells of the fresh, intact sclerotium. Annulment of soil fungistasis may be possible either by adding external nutrients (11,13) or by turning the hitherto unavailable internal sclerotial nutrients into available ones. A partial heat treatment was designed to cause a shock to a part of the sclerotium only, leaving most of the sclerotium protected inside the agar medium. The shock triggered an eruptive germination in the absence of WT-6 and rendered the sclerotia susceptible to the antagonist in field soil in a way that resembled that observed following the metham-sodium and drying treatments. Germination of the partially heated sclerotia took place at or near the treated area only.

The effect of metham-sodium on the sclerotia deserves special attention. In water solution, this chemical exhibits a relatively low toxicity to fungi. On the other hand, in soil it undergoes decomposition, releasing the highly fungitoxic methylisothiocyanate (MIT) (19). This explains the different results obtained with metham-sodium applied in water (Table 3) and soil. However, both treatments increased sclerotial susceptibility to WT-6. In contrast, NaClO alone induced noneruptive germination without

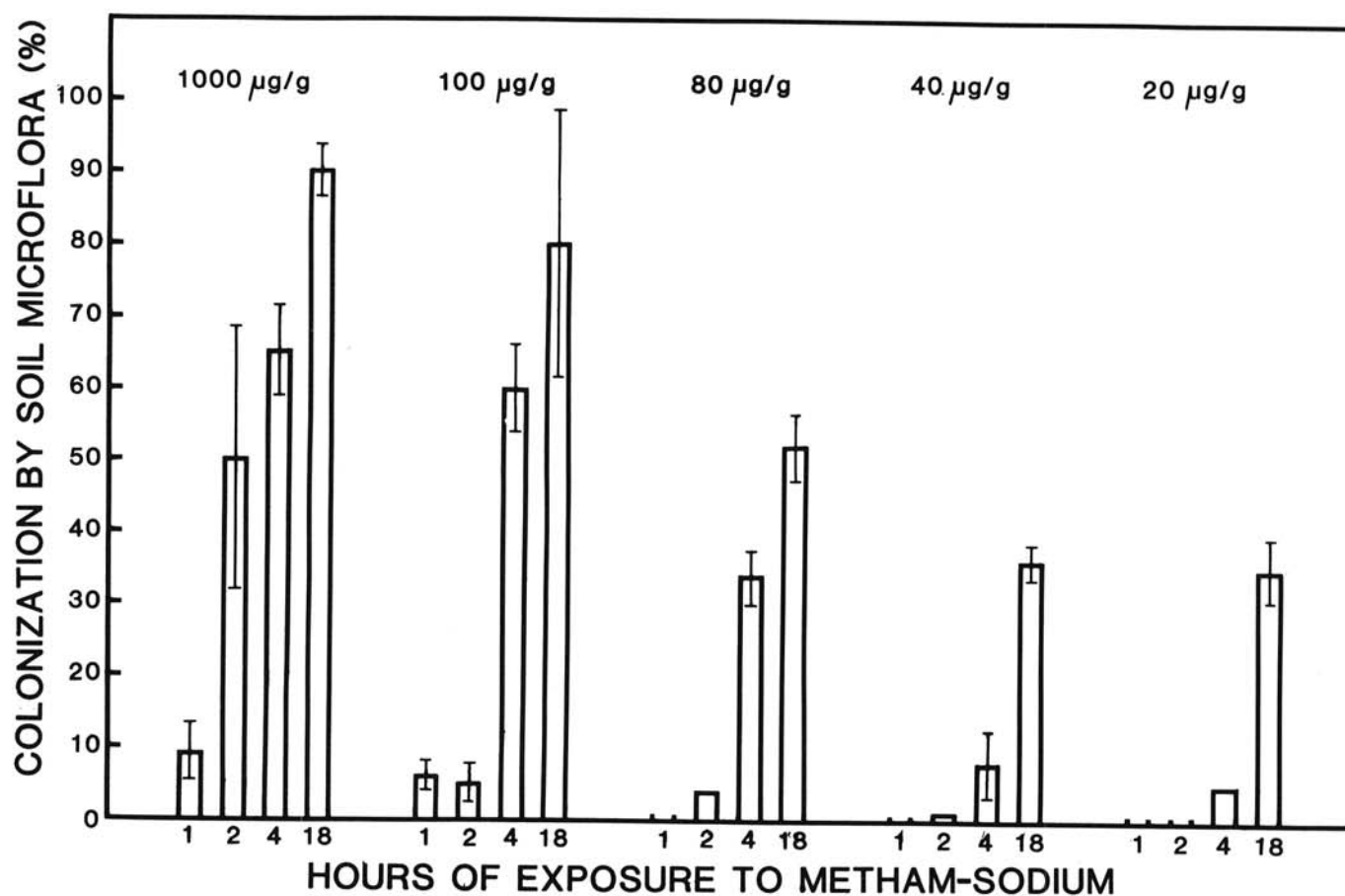


Fig. 3. Effect of metham-sodium pretreatment in soil on susceptibility of sclerotia of *Sclerotium rolfssii* strain Sr-3 to colonization by indigenous soil microflora. Pretreatments were done in soil vials. Washed sclerotia were then transferred to soil plates and incubated for 7 days at 28 C. Water controls of fresh sclerotia showed 0% colonization. Sclerotia were considered colonized when they showed visible growth and sporulation of alien fungi on their surface.

affecting sclerotial susceptibility to *T. harzianum*. This confirms the effect of NaClO on sclerotial germination first reported by Linderman and Gilbert (11). Although the overall effect of metham-sodium in soil depended on the rate of MIT release, there was no doubt as to the relationship between the effects of this chemical and subsequent infection by WT-6. The relationship between the *Trichoderma* and metham-sodium effects was demonstrated by the fact that *Trichoderma* alone did not degrade fresh viable sclerotia; metham-sodium at 1,000 µg/ml for 1 hr reduced viability by 22%; both treatments reduced viability by 80% (Fig. 2). Linderman and Gilbert (10-12) and others (1,15) showed that volatile substances from organic residues in soil stimulated sclerotial germination. Linderman and Gilbert (10-12) also showed that sclerotia were degraded by resident soil microbiota. Our results are in agreement with these reports as we showed that short exposure of sclerotia of *S. rolfii* to metham-sodium predisposed them to attack by the resident microbiota as well as by inoculum of *T. harzianum*. It is not known, however, whether increased germinability and predisposition to attack are related, and whether organic volatiles, MIT from metham-sodium, and heat shock have a common denominator. Possibly, the fungitoxic effect of metham-sodium under field conditions is also augmented by the natural soil microbiota (Fig. 3). Whether such effect really takes place is not known. However, it was shown here for the first time that these combined effects of metham-sodium and microorganisms in the soil can be greatly enhanced by treatment of the pathogen with spore suspensions of *Trichoderma* (Fig. 2).

We suggest that the common denominator of all the pretreatments in this research that rendered the sclerotia susceptible to *T. harzianum* was the turning of the hitherto unavailable food base into an available one. Whether this food

base is composed of dead tissue alone, leaking nutrient, activated living cells that respond to a shock by synthesizing specific proteins (8) with increased enzymatic activity, or both, remains to be studied. Lifshitz (9) applied heat treatment (47 C for 3 hr) to sclerotia of *S. rolfii* in water and in moist soil and demonstrated an increase in susceptibility to attack by *Aspergillus fumigatus*. He was unable to show whether *A. fumigatus* could attack an actively metabolizing mycelium of the pathogen, whether all treated sclerotial cells were equally affected, or whether only the dead sclerotial cells were involved. He did demonstrate, however, a synergistic effect of heat treatment and the antagonist on both germination and pathogenicity. We also suggest that in our tests a combined effect existed between biological and chemical factors on the one hand (Table 1) and between biological and physical factors on the other (Figs. 1 to 3). It is apparent that strain WT-6 required a food base for its attack and destruction of sclerotia and that both the pathogen and the antagonist competed for the same food base for germination and attack, respectively. This food base was not available to either of them in the fresh sclerotium. Chemical and/or physical pretreatments made this food base available to both the pathogen and the antagonist.

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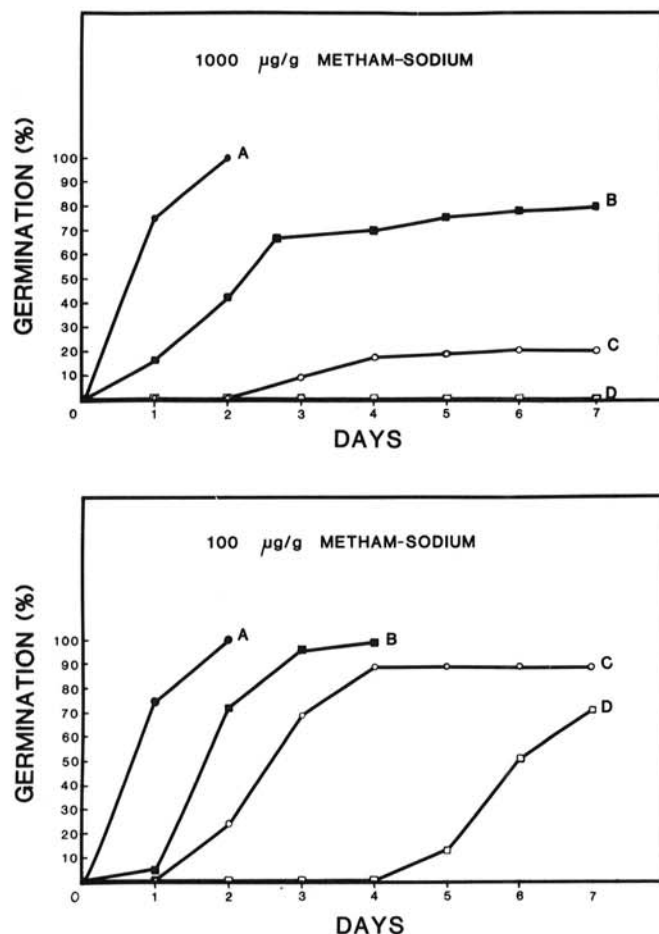


Fig. 4. Effect of metham-sodium pretreatment in soil on the germination pattern of sclerotia of *Sclerotium rolfii* strain Sr-3 on potato-dextrose agar-8-hydroxyquinoline medium. A, No metham-sodium; B, C, and D, 1, 2, and 4 hr pretreatment with metham-sodium, respectively.