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Prevention, with *Trichoderma harzianum* Rifai aggr., of reinfestation by *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kühn of soil fumigated with methyl bromide, and improvement of disease control in tomatoes and peanuts

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Abstract. Application of *Trichoderma harzianum* Rifai aggr. after soil fumigation with methyl bromide improved the control of *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kühn in a peanut field. Although soil fumigation controlled the diseases caused by these pathogens, it was followed by rapid reinfestation by *S. rolfsii* and *R. solani*. The biological control agent *T. harzianum* prevented reinfestation of the fumigated soil by the pathogens (88% reduction) both in a controlled environment and in field conditions. In soil treated with *T. harzianum*, survival of sclerotia was considerably less than in the untreated control. The combined treatment, of fumigation and *T. harzianum* applications, caused almost total mortality of sclerotia in soil in the laboratory and in the field. Application of *T. harzianum* to the root zone of tomatoes effectively controlled *S. rolfsii* in a field naturally infested with *S. rolfsii* and *R. solani*. Transplanting plants treated with *T. harzianum* into soil fumigated with methyl bromide reduced disease incidence by 93% and increased yield by 160%.

Introduction

Sclerotium rolfsii Sacc. is a facultative parasite of the stem bases and shoots of over 200 plants (Aycock, 1966). It is of economic importance on various legumes and numerous other cultivated plants including tomatoes, potatoes and ornamentals (Domsch, Gams and Anderson, 1980). In peanuts the disease caused by *S. rolfsii* may destroy the plants completely, leading to a rot of the pods and substantial losses of yield (Jackson and Bell, 1969; Rodriguez-Kabana, Backman and Williams, 1975). *Rhizoctonia solani* Kühn also is an important pathogen of peanuts. A significant

reduction in crop loss caused by these pathogens can be obtained by maintaining a low inoculum potential for several months. Fungicides such as pentachloronitrobenzene (PCNB) can be effective in controlling *R. solani* and *S. rolfsii* in peanut and tomato (Harrison, 1961). Soil fumigants such as methyl bromide are widely used to control a broad spectrum of soil-borne plant-pathogenic fungi including those mentioned above (Rankin and Good, 1959; Harrison, 1961; McCarter, Jaworski, Johnson and Williamson, 1976; Rodriguez-Kabana, Beute and Backman, 1979). Solar heating of the soil also gives an effective control of *S. rolfsii* in peanuts (Grinstein, Katan, Abdul Razik, Zeydan and Elad, 1979).

During the last few years we have shown, in Israel, that the biological control of soil-borne plant pathogens is feasible (Elad, Chet and Henis, 1981b). A wheat-bran preparation of an antagonistic fungus, *Trichoderma harzianum* Rifai aggr., applied to fields at rates of 500–1500 kg/ha, reduced the incidence of diseases caused by *S. rolfsii* and *R. solani* and this control led to increased yield in various crops (Chet, Hadar, Elad, Katan and Henis, 1979; Grinstein, Elad, Katan and Chet 1979; Elad, Chet and Katan, 1980a; Elad, Katan and Chet, 1980b; Elad *et al.*, 1981b; Elad, Hadar, Hadar, Chet and Henis, 1981c). The dose of *T. harzianum* could be reduced by direct application of this biological agent to the root zone of carnations. This method controlled *R. solani* as effectively as did the broadcast application of the biocontrol agent over the rows (Elad *et al.*, 1981b).

An integrated treatment comprising either PCNB, methyl bromide or soil solarization, combined with *T. harzianum*, resulted in a significant improvement in disease control over each treatment alone (Chet *et al.*, 1979; Elad *et al.*, 1980a,b). This *Trichoderma* species is now under development as a biological control agent for practical use, in the Hebrew University of Jerusalem, Rehovot, Israel.

Trichoderma spp. are the most common antagonists to appear after soil fumigation because they have few competitors and can reproduce rapidly (Munnecke, Kolbezen and Ohr, 1981). On the basis of this observation, the purpose of the work described in this paper was to achieve an efficient control of soil-borne pathogens, integrating fumigation and *Trichoderma* application. We have studied the ecology of *T. harzianum* and its ability to colonize soil that has been fumigated with methyl bromide; this antagonistic fungus prevents soil reinfestation by pathogens, and may lead to long-term disease control.

Materials and methods

An isolate of *Trichoderma harzianum*, which is antagonistic towards both *Sclerotium rolfsii* and *Rhizoctonia solani* (Elad *et al.*, 1980a,b; 1981a,b) was grown on autoclaved wheat bran and was used throughout this study. Both field and greenhouse experiments were conducted in a soil which consisted of 30% sand, 18% silt, 49% clay and 3% organic matter, pH 7·95, with a moisture-holding capacity of 39%. All field experiments were carried out in six replicates arranged in a randomized block design. Laboratory, growth chamber and greenhouse experiments were repeated three times.

Laboratory experiments

Either soil (with a 10% moisture content, maintained by adding water every week), or sclerotia of *S. rolfsii*, were fumigated with 200 mg/kg of Methabrom (98% methyl

bromide, supplied by Bromine Compounds, Beer-Sheva, Israel). Sclerotia, prepared according to the method described by Elad *et al.* (1980a), were mixed with soil at a rate of five sclerotia per gram of soil. *T. harzianum* wheat-bran preparation containing 5×10^9 colony-forming units per gram was also mixed with soil. Ten grams of soil from each of these treatments was then placed in 15 glass tubes which were incubated in an illuminated chamber at 28°C ± 1°C for up to 200 days. On each sampling day, soil from three tubes of each treatment was aseptically spread on a metal mesh (500 μm grid) which was placed on a funnel and washed with 100 ml tap water into a 250 ml beaker. This procedure separated the sclerotia from the soil. The washed soil sediment was used for the determination of *Trichoderma* spp. propagules on *Trichoderma*-selective medium (TSM) as described by Elad *et al.* (1981a). Sclerotia were aseptically placed on agar plates containing a synthetic medium (SM) prepared according to the method of Okon, Chet and Henis (1973) and supplemented with 0·25 g chloramphenicol (Chloromycetin, Sigma Chemical Co., USA), 0·2 g sodium 4-dimethylaminobenzenediazosulfonate (fenaminosulf; Dexon 60% w.p., Farbenfabrik Bayer A.G., Germany), and 0·1 g bromocresol green (May & Baker, England), per litre of distilled water. The percentage of sclerotia from which fungi emerged after 3 days' incubation on SM at 30°C was recorded.

Greenhouse experiments

Reinfestation after fumigation. Reinfestation, by *S. rolfsii*, of fumigated soil was studied in a growth chamber at 28°C ± 3°C using plastic boxes measuring 10 × 20 × 30 cm. All experiments comprised six replications. Soil samples were subjected to one of the four following treatments: (1) untreated; (2) *T. harzianum* application; (3) fumigation with methyl bromide (0·2 g/kg); (4) fumigation followed by *T. harzianum* application. A sample was placed in one half of a box, the other half being filled with soil which had been infested artificially with *S. rolfsii* (0·2 g sclerotia/kg soil). Four rows each of six beans (*Phaseolus vulgaris* L., cv. Brittle Wax) were planted in both parts of each box. Disease symptoms caused by *S. rolfsii* were recorded for plants in each row, throughout the experiment. Soil from the four treatments, which previously had been devoid of *S. rolfsii*, was transferred after 33 days into boxes measuring 10 × 10 × 6 cm, and sown with beans four times in succession, in order to study the potential of *Trichoderma* spp. to prevent subsequent build-up of disease caused by *S. rolfsii* (Elad *et al.*, 1980b).

Inoculum potential of R. solani *and* S. rolfsii. The inoculum potential of the general population of *R. solani* and *S. rolfsii* in soil from field trials was estimated using beans as a test plant. Nine seeds of *Phaseolus vulgaris* were sown in plastic boxes measuring 10 × 10 × 6 cm, which contained samples of soil taken from peanut field experiments (*see* Field experiments – peanuts). The boxes, arranged in seven replicates, were maintained in the greenhouse at 24–30°C for 28 days, when the number of diseased plants was recorded. At the end of this experiment, all remaining plants were uprooted and examined for the presence of either pathogen by plating-out plant fragments on SM plates.

T. harzianum *in tomato seedling rooting media.* Tomatoes (*Lycopersicon esculentum* L., line M-82) were rooted by the 'Speedling' method (Elad *et al.*, 1981c). The rooting media were peat alone, or peat plus 15% (v/v) *T. harzianum* preparation for

application to the root zone. These media were placed in 'Speedling'-type preformed trays (30 × 90 cm), which were divided into conical compartments with one tomato seed planted in each compartment (Elad *et al.*, 1981c). Three weeks later, individual seedlings were transplanted, together with the rooting media, into plastic boxes (10 × 20 × 30 cm) which were filled with soil naturally infested with *S. rolfsii*. These 'Speedlings' were maintained for 6 weeks in the greenhouse, after which the incidence of disease was recorded.

Field experiments

Peanuts. The field experiments were carried out in naturally infested soil in which peanuts and tomatoes had frequently been grown for the past 15 years. The experiments comprised six replicates arranged in a randomized block design. Peanut-field plots (3·6 × 10 m) were subjected to one of the following four treatments: (1) untreated; (2) fumigated with methyl bromide (50 g/m^2) on 17 April 1980; (3) treated by incorporating 50 g dry weight per m^2 of *T. harzianum* preparation on 29 April, to a depth of 8 cm using a rotary hoe; (4) methyl bromide fumigation plus *T. harzianum* application as described above. Peanut (*Arachis hypogaea* L. cv. Shulamit) seeds were planted, eight to a row, on 8 May. The furrows were drenched with peanut-specific *Rhizobium* preparation during sowing. All plots were treated with terbutryn herbicide (2-*tert*-butylamino-4-ethylamino-6-methylthio-1,3,5-triazine) at planting time. During the growing season, soil samples were taken for assessment of the inoculum density of *S. rolfsii* and of *R. solani* and numbers of *Trichoderma* spp. Plants that had been destroyed completely by soil-borne plant pathogens were removed and their numbers recorded. Remaining plants were harvested on 28 September, when they were uprooted from each plot and their roots and pods examined for disease symptoms. Because the experimental field soil was naturally infested with *S. rolfsii*, *R. solani* and *Verticillium dahliae* Kleb., the presence of these pathogens in diseased plants was verified by plating-out plant fragments on SM plates.

Sclerotia buried in peanut fields. Sclerotia of *S. rolfsii* taken from SM plates (Elad *et al.*, 1980a) were mixed with field soil in a 1 : 1 ratio (w/w) and the mixture was placed in plastic net bags which were then buried in the field plots at depths of 5 cm and 20 cm. The contents of these bags were sampled during the course of the growing season, and tested in the laboratory in the same way as described for the test-tube experiment (*see* Laboratory experiments).

Tomatoes. Tomato seedlings were prepared for field experiments by the 'Speedling' method, described above. Tomato seedlings, with roots either loaded or not loaded with *T. harzianum*, were subsequently transplanted in rows (10 plants/m) on 26 June into field plots (3 × 1·8 m) which either had been previously fumigated with methyl bromide (50 g/m^2) on 14 May, or had not been fumigated. Plant death caused by *S. rolfsii* (southern blight) was recorded during the growth period, and soil samples were taken. On 4 September 1980, tomato plants were uprooted, examined for symptoms of southern blight, and the yield of tomatoes determined.

Results

Survival of Trichoderma harzianum *and of sclerotia of* S. rolfsii *in fumigated soil (Laboratory experiments)*

Methyl bromide affects the sclerotia of *S. rolfsii* either directly, or indirectly through its effects on other members of the soil population. When the response of the sclerotia to methyl bromide fumigation was studied in test tubes under controlled conditions of 28°C and 10% humidity, for up to 200 days, a high percentage of untreated sclerotia survived both in fumigated and in non-fumigated soil (Table 1). However, fumigation of sclerotia reduced their viability. The natural *Trichoderma* spp. population, which was 5 propagules/g immediately after soil fumigation, increased in all treatments during the 200-day incubation period, as shown in Table 1. Intermediate sampling (data not given in Table 1) showed that, when *T. harzianum* was mixed with fumigated soil, then all fumigated sclerotia died within 45

TABLE 1. Survival of *Sclerotium rolfsii* sclerotia after 200 days' incubation, in relation to methyl bromide fumigation and population of *Trichoderma* spp. in soil (test-tube experiment)

Methyl bromide treatment of *S. rolfsii* sclerotia	−	+	−	+
Methyl bromide treatment of soil	−	−	+	+
200-day survival of sclerotia* (%)	75	0	55	0
S. rolfsii sclerotia from which *Trichoderma* spp. emerged (%)	3	16	11	80
Natural population of *Trichoderma* spp.† (P/g)	$1{\cdot}0\times10^2$	$6{\cdot}0\times10^2$	$2{\cdot}0\times10^3$	$2{\cdot}8\times10^5$

* Assessed by incubation of separate sclerotia on SM agar plates for 3 days.

† Determined by serial dilutions on TSM plates after 200 days' incubation of soil in test tubes (P/g=propagules per gram).

days of the start of incubation and *Trichoderma* emerged from 100% of sclerotia: however, if the soil had not been fumigated, then total mortality of fumigated sclerotia took up to 90 days (*Trichoderma* emerged from 80% of sclerotia). This indicates that *Trichoderma* parasitism is the major cause of death of sclerotia.

Prevention of reinfestation of fumigated soil (growth-chamber experiments)

When beans were grown in boxes of treated soil adjacent to soil infested with *S. rolfsii*, the disease incidence 33 days after planting was 18%, 80%, 2% and 12% in plants growing in untreated soil, fumigated soil, soil mixed with *T. harzianum*, and soil given the combined treatment, respectively (Figure 1a). Disease reduction was also noted near the edges of the infested soil, adjacent to the *Trichoderma*-treated soils (Figure 1b). During the experiment, *Trichoderma* was found in the neighbouring *S. rolfsii* infested soil, reaching levels higher by two to three orders of magnitude than those found on average in infested soils.

When the soils from these experiments were resown with beans four times in succession, symptoms of southern blight (caused by *S. rolfsii*) were reduced by 40%

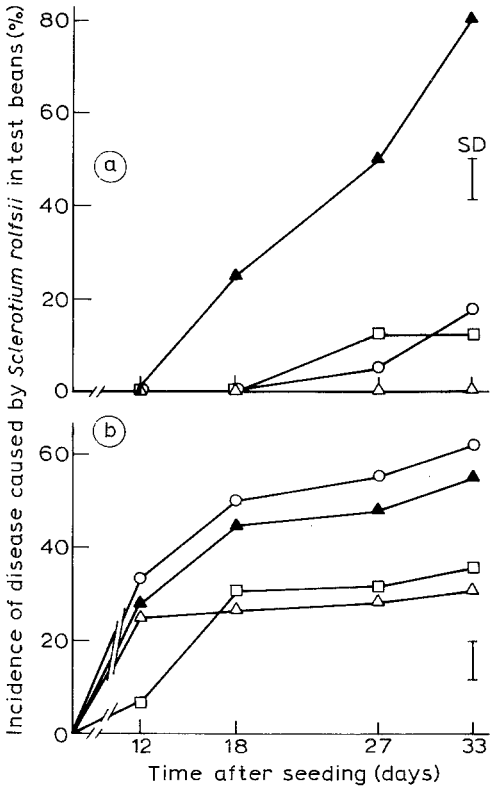

FIGURE 1. Reinfestation, by *Sclerotium rolfsii*, of treated soil in plastic boxes. One half of each box was filled with soil infested artificially with *S. rolfsii* (0·2 g sclerotia/kg soil) while the other half of each box was filled with soil which had received one of the following treatments: untreated control (○); *Trichoderma harzianum* application (△); methyl bromide fumigation (▲); combined treatment with *T. harzianum* and methyl bromide (□). The percentage incidence of diseased test bean plants in the treated soil (a) or in the border region (5–7 cm wide) of the infested soil adjacent to the treated soil (b) was recorded.

in the *T. harzianum* treatments compared with the plants in the untreated soil. The *T. harzianum* population in the *Trichoderma*-treated soil samples declined from 5×10^6 propagules (P)/g at the beginning of the experiment to 7×10^4–3×10^5 P/g at the end of the fourth planting.

Inoculum potential of pathogens in field soils

In beans growing in samples of soil from peanut-field plots, where no treatment had been given to the soil, damping-off symptoms caused by *R. solani* gradually developed so that eventually 77% of the test bean seedlings were infected, whereas southern blight symptoms caused by *S. rolfsii* were recorded for 41% of plants. However, the incidence of damping off and southern blight in the *Trichoderma*-treated plots was only 50% and 17% respectively (Figure 2a, b). In fumigated plots,

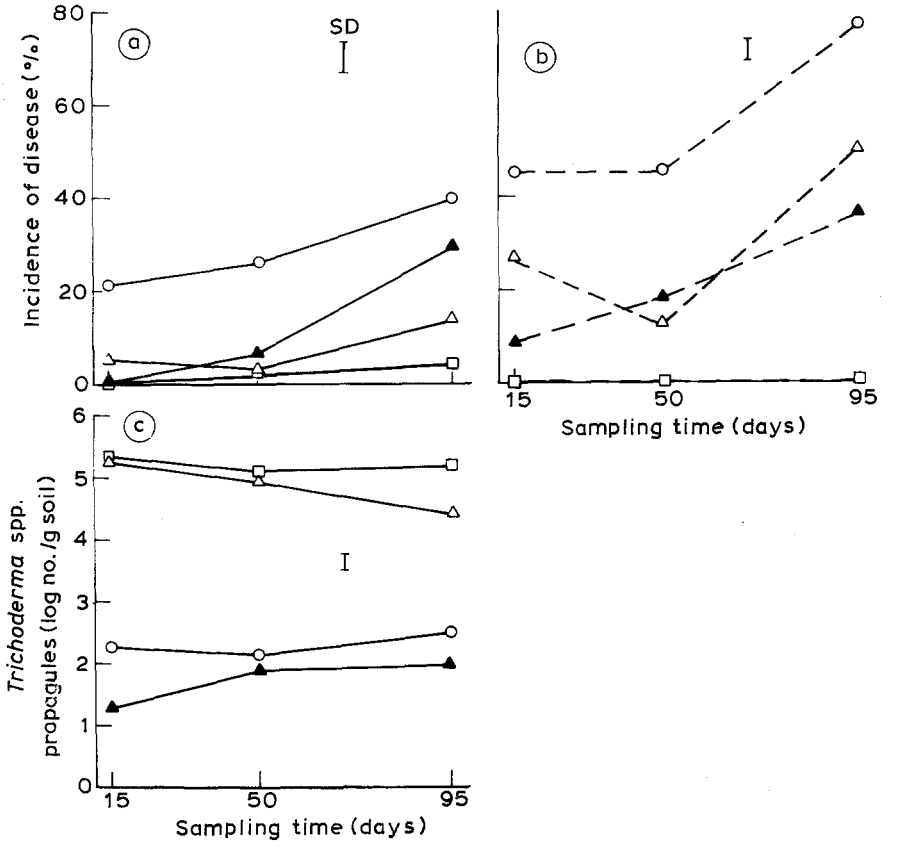

FIGURE 2. Incidence of disease caused by (a) *S. rolfsii* or (b) *R. solani* in beans grown in soil samples from centre of trial plots from peanut field; (c) population of *Trichoderma* spp. in this soil. Soil treatments: ○ untreated control; △ *Trichoderma harzianum* application; ▲ methyl bromide fumigation; □ combined treatment with *T. harzianum* and methyl bromide.

the respective incidence of the two diseases was 37% and 30%, whereas in soil receiving the combined treatment, the incidence of disease caused by either pathogen did not exceed 3% (Figure 2a, b).

The *Trichoderma* sp. population in the untreated soils ranged from $1{\cdot}5 \times 10^2$ to $3{\cdot}2 \times 10^2$ P/g during the growth period. However, in *Trichoderma*-treated plots, *T. harzianum* counts were $3{\cdot}0 \times 10^5$ P/g at the beginning of the growing season and $2{\cdot}3 \times 10^4$ P/g 80 days later (Figure 2c). In untreated soil and in soil given methyl bromide fumigation alone, the *Trichoderma* count 95 days after peanuts were planted was $2{\cdot}5 \times 10^2$ and $1{\cdot}0 \times 10^2$ P/g respectively, whereas after the combined treatment, the soil population was $2{\cdot}8 \times 10^5$ P/g.

Soil samples were taken from different sites in the field plots, in order to examine the ability of *T. harzianum* to prevent reinfestation of a plot from the adjoining untreated soil. Test plants grown in soil taken from either the edges or the centre of untreated field plots showed no difference in the incidence of symptoms of damping off (*R. solani*) nor of southern blight (*S. rolfsii*). On the other hand, when samples taken from the edges of *Trichoderma*-treated plots (with or without methyl bromide

fumigation) were compared with those taken from the centre of these plots, an increase in disease incidence of 200% (combined treatment) and 50% (*Trichoderma* treated alone) was noted, i.e. the centres of the plots were less infested than the edges. This is in contrast to the results from plots which received fumigation but not *Trichoderma* treatment, in which infection by both pathogens was 30% lower at the edges compared with the centre of the plots. These results indicate that the use of *Trichoderma* prevented the reinfestation of the centre of the plots which occurred in the fumigated soil.

In fumigated plots (with or without *T. harzianum*), the population of *Trichoderma* spp. was 7–10 times higher at the edges of the plots, compared with the centre. This pattern of variability in *Trichoderma* level was not observed in the other two treatments (untreated, and *T. harzianum* alone).

Integrated control of soil-borne pathogens of peanuts under field conditions

When the peanut plants grown in the 1980 field experiment were uprooted at harvest, and their roots and pods were examined for the presence of symptoms caused by soil-borne plant pathogens, the number of plants with southern blight symptoms (caused by *S. rolfsii*) was found to have been reduced by 49% by either methyl bromide fumigation or by *T. harzianum* treatment, and by 71% by a combination of both treatments. Similarly, symptoms of disease caused by *R. solani* or by *Verticillium dahliae* were reduced by either treatment alone, and by the combined treatment (the latter resulting in the lowest incidence of disease, *see* Table 2). The percentages of healthy peanut pods were 42% from untreated plots, 81·5% from fumigated plots, 69·0% from *Trichoderma*-treated plots and 91% from those plots given the combined treatment. Yields from the methyl bromide, *T. harzianum* and combined treatments were, respectively, 20%, 17% and 58% higher than the yield of 8920 kg/ha from the untreated plot. These results are similar to those from an experiment carried out in the same region one year previously (unpublished data).

TABLE 2. Severity of soil-borne fungal disease in a peanut field (1980)

Treatment	*Sclerotium rolfsii*		*Verticillium dahliae*	*Rhizoctonia solani*
	Number of diseased plants per 100 m row	Diseased plants in the centre of the plot (%)	Number of diseased plants per 100 m row	
Untreated control	163 a*	52 a	97 a	433 a
T. harzianum (TH) (500 kg/ha)	83 b	38 b	27 b	323 b
Methyl bromide (MB) (500 kg/ha)	83 b	36 b	53 b	296 b
MB+TH	48 c	22 c	10 c	260 b

* Numbers followed by a common letter are not statistically different according to Duncan's multiple range test ($P \leq 0{\cdot}05$).

Survival of S. rolfsii *sclerotia buried in field plots*

The viability of *S. rolfsii* sclerotia, which had been mixed with field soil and placed in plastic net bags which were buried in the field plots, was lowest in the plots previously treated with both *Trichoderma* and methyl bromide (Figure 3a). In the untreated and in the *Trichoderma*-treated soil, sclerotial viability at a depth of 5 cm did not differ significantly or consistently from that at 20 cm. However, in fumigated soil, survival of sclerotia was 45% at a depth of 5 cm, but only 25% at 20 cm. Colonization of sclerotia by *Trichoderma* was significantly higher, by 20–40%, in the *Trichoderma*-treated soils (Figure 3b). In the control or fumigated plots, 80–89% of dead sclerotia lifted contained various types of *Trichoderma* spp., whereas the antagonist was isolated from *all* the dead sclerotia found in *Trichoderma*-treated soil.

In the soil surrounding the buried sclerotia, by the end of the peanut-growing period, the *Trichoderma* population had increased to 10^3 and 5×10^6 P/g in the control and in the *Trichoderma*-treated plots, respectively (Figure 3c). In the fumigated soil, with a *Trichoderma* population of 10^3 P/g, the counts of *Trichoderma* surrounding the sclerotia buried at a depth of 20 cm (data not shown) were 60–90 times greater than in those buried at a depth of 5 cm.

In an additional experiment, the sclerotia which were lifted from the field plots were also mixed with soil which was sown with beans and kept in the greenhouse. Sclerotia which had been buried in fumigated plots for 95 days, infected about 25% of the test bean seedlings with southern blight. In those sclerotia which were buried in plots given the combined *T. harzianum* plus fumigation treatment, the capability of the sclerotia to infect beans fell to 0–5% after 95 days' burial (Figure 3d).

Application of Trichoderma *preparations to the root zone of tomatoes (greenhouse and field experiments)*

Individual tomato seedlings were rooted in preformed trays containing peat-filled compartments measuring either 3×3 cm or 6×6 cm, before being transplanted into boxes of soil naturally infested with *S. rolfsii* and maintained for 6 weeks in the greenhouse. Disease incidence in the untreated plants grown in the smaller compartments was 42% while that of plants grown in the larger compartments was 24%. In plants where *Trichoderma* had been applied to the root zone, the corresponding disease incidence was 22% and 14%.

In a field experiment, the effects of a combination of methyl bromide fumigation with *Trichoderma* treatment of the root zone were examined. Application of *T. harzianum* to the root zone increased the population of this antagonistic fungus in soil (Table 3). The survival of the tomato seedlings was improved by all treatments compared with the control, the highest survival rate being given by the combined treatment. *R. solani* and *S. rolfsii* were undetectable immediately after soil fumigation, whereas at the end of the growth period they were found both in fumigated and in non-fumigated plots (Table 3).

The incidence of symptoms of *S. rolfsii* on tomato plants under field conditions was reduced by all treatments. However, the best control (93% reduction in incidence of symptoms) was achieved by the integration of treatments with methyl bromide and *T. harzianum*. This integrated treatment gave the highest increase in yield of tomatoes – 160% (Table 3).

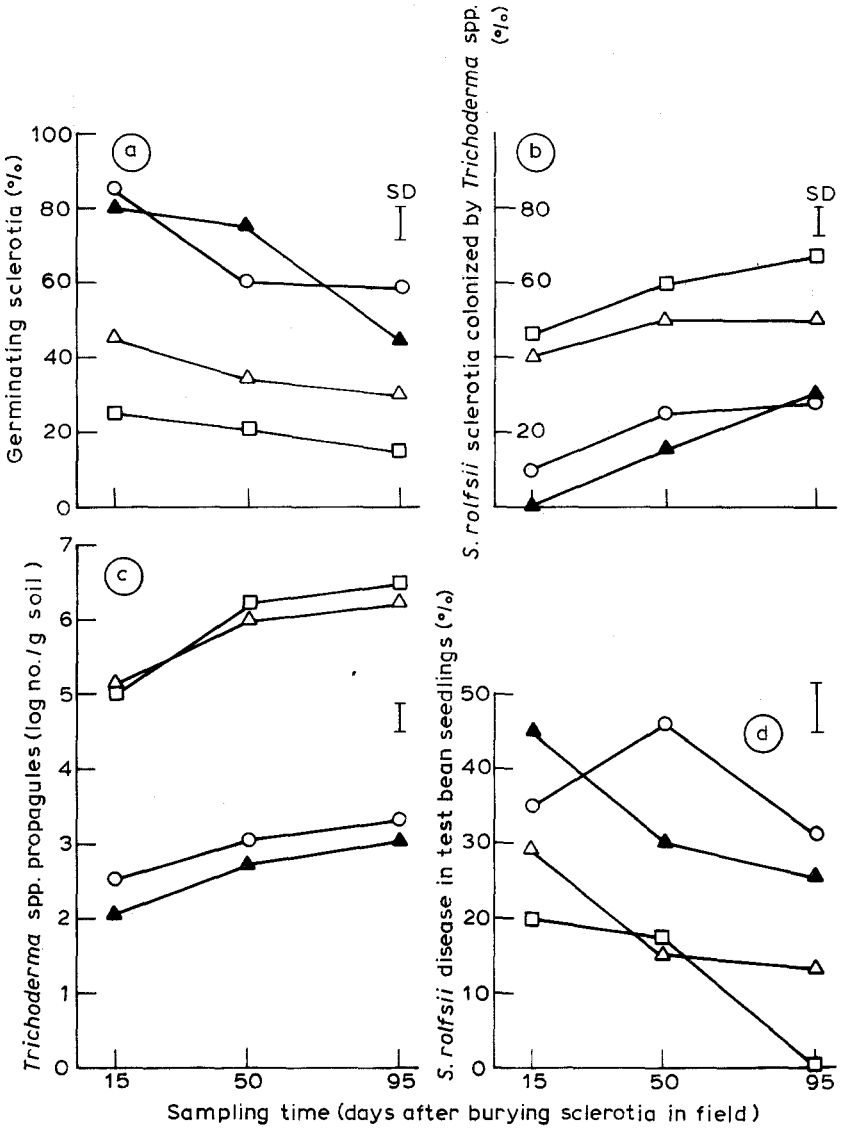

FIGURE 3. Changes in various characteristics of sclerotia of *S. rolfsii* which were buried to a depth of 5 cm in peanut-field plots that had been treated as follows: ○ untreated control; △ *Trichoderma harzianum* application; ▲ methyl bromide fumigation; □ combined treatment with *T. harzianum* and methyl bromide. (a) Sclerotial germination (viability). (b) Percentage of *S. rolfsii* sclerotia colonized by *Trichoderma* spp. (c) Population of *Trichoderma* spp. in the soil surrounding the buried sclerotia. (d) Incidence of disease, in test bean seedlings, caused by *S. rolfsii* sclerotia lifted from field plots.

TABLE 3. The effect of soil fumigation with methyl bromide and *T. harzianum* application to the root zone on *R. solani* and *S. rolfsii* disease, seedling establishment and yield of tomatoes

Treatment in field	*T. harzianum* application to root zone	*Trichoderma* spp. population (propagules/g soil)*	Survival of seedlings in field (%)	Inoculum potential* (expressed as % diseased bean seedlings in greenhouse tests)		Tomato plants infected with *S. rolfsii* (%)	Yield of tomatoes (kg/ha)
				R. solani	*S. rolfsii*		
Non-fumigated soil	–	150	70 a†	47 c	19 b	22 c	4510 a
	+	2250	79 ab	33 b	10 a	12 b	7070 b
Methyl bromide (500 kg/ha)	–	1520	81 b	55 c	17 b	7 b	6520 b
	+	7100	98 c	13 a	17 b	1·5 a	11770 c

* Soil samples, taken between the tomato plants 53 days after transplanting into the field, were assessed for *Trichoderma* population or planted with beans and incubated in the greenhouse.

† Numbers followed by a common letter are not statistically different according to Duncan's multiple range test ($P \leq 0{\cdot}05$).

Discussion

Although soil disinfestation with methyl bromide is an effective way in which to control severe fungal diseases, including those caused by *Sclerotium rolfsii* and *Rhizoctonia solani* (Harrison, 1961; McCarter *et al.*, 1976; Elad *et al.*, 1980a), nevertheless, reinfestation of the fumigated soil often occurs. The degree of reinfestation is proportional to the reduction in antagonistic micro-organisms and the alteration of the original ecosystem which follows disinfestation. Eradicants, such as methyl bromide, cause a 'microbial vacuum' which may soon be followed by a build-up in the population of pathogens (Kreutzer, 1965). This effect was noted in our work with *S. rolfsii* in fumigated soil which was subsequently kept in contact with infested soil. In the fumigated soil, disease incidence in test bean seedlings increased to 80%, compared with only 18% in the untreated soil (Figure 1a).

A rapid build-up of *Trichoderma* isolates in soils treated with chemicals has already been reported by Mughogho (1968). In the work reported here (Table 1) a 20-fold increase in the population of *Trichoderma* spp. was found in fumigated infested soil, compared with that in unfumigated infested soil. After fumigation, *Trichoderma* spp. can tolerate low doses of methyl bromide (Munnecke, Kolbezen and Wilbur, 1973; Ohr, Munnecke and Bricker, 1973). However, the natural increase of *Trichoderma* spp. following fumigation did not result in biological control of *S. rolfsii*; artificial introduction of the biological control agent into soil is therefore required. In this way, *T. viride* has been applied to soil fumigated with methoxyethyl mercuric chloride, to prevent reinfestation of tree roots by *Phytophthora cinnamomi* (Marques and Touvet, 1972). In our work, the shift in biological equilibrium of the fumigated soil, as manifested by the high level of the biological control agent, prevented disease build-up, both in growth chambers (88% reduction) and in the field (Figures 1a, 2a, 2b).

The combined treatment of fumigation with *T. harzianum* application killed all sclerotia in soil, under laboratory conditions, and in the field the percentage of sclerotial kill was much higher than in *Trichoderma*-treated unfumigated soil (Figure 3a). It has already been noted that buried sclerotia of *S. rolfsii* tolerate a very high concentration of methyl bromide under field conditions (Munnecke, Bricker and Kolbezen, 1978). This is in agreement with our data which show that not all the sclerotia were killed by the fumigant (Figure 3d). However, the pesticide apparently weakened the sclerotia so that susceptibility to *T. harzianum* was increased (Figure 3b).

In the peanut field, fumigation reduced the incidence of diseases caused by *R. solani* and *S. rolfsii* by 52% and 27% respectively. *T. harzianum* alone reduced the diseases by 35% and 59% respectively, whereas with the combined treatment a total control of these diseases was obtained (Figure 2a, b).

Examination of reinfestation patterns of *R. solani* and *S. rolfsii* showed that 30% more infestation occurred at the centre of the fumigated plots than at the edges, and that invasion of both pathogens into the centre of the field plots was significantly reduced in the soil treated with *T. harzianum*. In the fumigated plots, an increased population of *Trichoderma* spp. was found at the edges and at a depth of 20 cm: at such sites fumigation is usually less efficient, thus enabling the relatively resistant *Trichoderma* to develop.

Application of *T. harzianum* to the roots of tomato plants before transplantation into fumigated field soil, gave superior results to those from either fumigation or *T. harzianum* treatment alone, both in *S. rolfsii* disease reduction and in yield increase (Table 3). These results are similar to those reported for *R. solani* in carnations (Elad *et al.*, 1981c). This method has the advantage of using high concentrations of *T. harzianum* at the infection site, while the total amount applied to the field as a whole is very small.

In the work described here we have investigated different approaches for the integrated control of both *R. solani* and of *S. rolfsii*. We have found that the efficiency of fumigation is increased by subsequent application of *Trichoderma* preparations, indicating that such a combination of control methods may enable smaller doses of methyl bromide to be used, and may result in longer-lasting control of soil-borne diseases in the field.

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