Control of *Rosellinia necatrix* in Soil and in Apple Orchard by Solarization and *Trichoderma harzianum*

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ABSTRACT

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Various types of natural or culturally produced inocula of *Rosellinia necatrix* (anamorph: *Dematophora necatrix*) are highly sensitive to heat; 50–100% mortality was recorded after 4 hr at 38 C. Inoculum of the pathogen in inoculated wheat seeds became less heat-sensitive after aging. In one of two soils, combining sublethal heating in the laboratory with application of *Trichoderma harzianum* further improved pathogen control over that achieved by either treatment alone. Solarization by polyethylene tarping of the soil resulted in effective control of the pathogen and disease (as assessed by various methods). Partial or complete pathogen control was obtained in tarped-shaded (unheated) plots. Growth of the pathogen was reduced in a previously solarized soil, as assessed by leaf colonization and mycelial growth methods, thus indicating soil suppressiveness. Soil solarization of an existing orchard with infected apple trees reduced disease considerably over at least 25 mo. *T. harzianum* was not effective in disease control under the tested field conditions.

Additional key words: avocado, integrated control, soil mulching, solar heating

Rosellinia necatrix Prill. (anamorph: *Dematophora necatrix* Hartig) is the causal agent of the white root rot disease of many plants, particularly of fruit trees such as apple, pear, and avocado (13,21). The symptoms are rotting of roots, yellowing of leaves followed by leaf fall, wilting, and death of the tree. Usually, the infected roots are covered with white mycelium.

Various approaches that might be followed for the control of *R. necatrix* are: 1) eradication of the fungus in infested soils by soil disinfestation to prevent spread of the pathogen to adjacent fields, 2) suppression of the pathogen in soil and, therefore, protection of the plant from infection, and 3) treatment of the soil in an existing orchard where trees are diseased to prevent further infection. Both solarization, i.e., solar heating of the moistened soil by tarping with transparent polyethylene (12), and the antagonistic fungus *Trichoderma harzianum* Rifai (6) are potential control measures for use against *R. necatrix* and other soilborne pathogens. The purpose of this work was to study the effectiveness of artificial heating of inocula, solarization, and *T. harzianum* treatments, separately and combined, for control of *R. necatrix*

under controlled and environmental conditions, in a field soil and in an existing orchard.

MATERIALS AND METHODS

Pathogen. *R. necatrix* was isolated from roots of naturally infected apple (*Malus sylvestris* Mill. seedling rootstock) from Kibbutz En Zurim in the south and from naturally infected roots of avocado (*Persea americana* Mill.) from Kibbutz Hanita in the north of Israel. In both cases, the trees showed symptoms typical of the disease, i.e., chlorosis, leaf fall, and dieback.

Antagonist. *T. harzianum* was isolated from roots of an apple tree naturally infected by *R. necatrix* from Kibbutz En Zurim. The fungus was grown for 2 wk on a wheat bran/peat (1:1, v/v) preparation (19). Various quantities (1 and 5 g) of this preparation were mixed with naturally infested En Zurim soil, Vertisol (3.7% silt, 46.3% clay, and 50% sand; pH 7.6), or Hanita soil, Terra rosa (17.5% silt, 55% clay, and 27.5% sand; pH 7.8), to a *T. harzianum* population of 10^8 colony-forming units per gram dry weight of soil mixture.

R. necatrix inoculum. Inoculum was produced in wheat seeds, which were soaked for 12 hr in 250-ml Erlenmeyer flasks filled with distilled water. The flasks, each containing 100 ml of seed, were subsequently autoclaved after excess water had been drained off. After sterilization, three fungal disks of a 2-wk-old culture of *R. necatrix* grown on malt-extract agar (MEA) were placed aseptically in each flask. Flasks were then incubated at 25 C in light for 2 wk, unless otherwise stated, and shaken every 2–3

days to avoid clustering of seeds. The inoculum in each flask was then macerated in a Waring Blendor under sterile conditions and a 4-g quantity was mixed with 1 kg of sterile autoclaved En Zurim soil.

Inoculum level assessment. The avocado leaf disk colonization method was used to determine inoculum levels of *R. necatrix* (23) in naturally and artificially infested soils. This method is reliable for assessing relative levels of the pathogen population, and within certain limits, inoculum density and percentage of colonization are linearly related (6). The tested soil was placed in plastic containers ($11 \times 11 \times 4$ cm) each holding 250 g of soil, with avocado leaf disks (1.6 cm diameter) serving as traps for *R. necatrix*. These containers were incubated in light at 25 C for 12–14 days, then leaf colonization was assessed. The colonized disks developed characteristic white mycelium and changed color to cream or light brown, whereas noncolonized disks were dark brown or remained green. All treatments were carried out in four replicates with 15 avocado leaf disks each. Percentage of reduction in colonization (C) was calculated by the following equation: $C = [(B - A)/B] \times 100$, where A = percentage of colonized disks in the treatment and B = percentage of colonized disks in the untreated control.

Heat treatment of R. necatrix inocula. Quantities of 100 g of soil either naturally infested or containing culturally produced inocula were placed in plastic bags (18×24 cm) and heated at constant temperatures in a plastic water bath. Inocula of *R. necatrix*, consisting of artificially infected wheat seeds at 2-, 4-, and 6-wk incubation periods and naturally infected apple root segments, were inserted into test tubes containing 10 ml of sterile water. The test tubes were capped and incubated at various temperatures for 0–4 hr in a water bath to determine heat sensitivity of inocula of various ages and types. After heat treatment, viability of the inocula was determined by monitoring mycelial growth from wheat seeds incubated 48–72 hr on MEA plates and from roots in moisture chambers at 25 C. Seed samples consisted of 40 seeds per treatment with 10 seeds per plate. Infected root samples consisted of 40 segments (1.5–2 cm) per treatment. Viability of inoculum in soil was assessed by the avocado leaf colonization method.

Control of _R. necatrix_ under field conditions. The field experiment was carried out in a 15-yr-old apple grove, in naturally infested soil at Kibbutz En
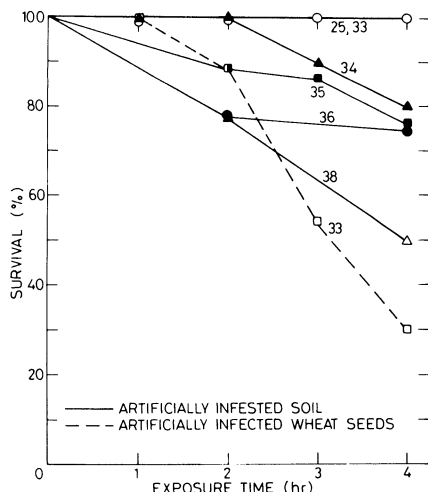

Fig. 1. Effects of heating on survival of _Rosellinia necatrix_ in either artificially infested soil or inoculated wheat seeds. Survival was determined in artificially infested soil by the leaf colonization method and in wheat seeds by the percentage of seeds yielding the pathogen after treatment. Numbers on the graph lines indicate temperature (C). Following a linear regression, all the coefficients ($-0.99 < r < -0.98$) are significant except for graph line indicating 36 C, and slopes are significantly different from zero (graph line indicating 25, 33 C) ($P = 0.05$).

Zurim. Treatments were: 1) soil solarization, 2) _T. harzianum_, 3) solarization combined with _T. harzianum_, and 4) an untreated control. Twenty plots were selected randomly with treatments consisting of five replicate plots. Each plot (10×6 m) contained one established tree and the area of an adjacent tree that had been killed by _R. necatrix_ and uprooted 2 mo before the experiment was begun. The area directly under the canopy of the existing tree (about 2 m²), which was not exposed to direct sunshine and was shaded during most of the day, is referred to as the tarped-shaded site. Trees in the orchard were spaced 3.5×4 m apart.

The treated plots were sprinkler-irrigated to a depth of 90–120 cm. The soil of 10 plots (five solar and five integrated solar- and _T. harzianum_-treated plots) was covered by 40-μm-thick, transparent polyethylene sheeting on 4 July 1984. It was removed 8 wk later. The solar-treated trees were situated 3 m from the edges of the tarped sheeting. Outer edges of the tarps were buried in shallow trenches to hold them in place. The existing trees in the tarped plots were irrigated by a drip system during and after solarization.

Soil temperatures were recorded with Grant equipment (United Kingdom) by means of thermistors for 2 wk, starting 12 days after solarization had begun, at

depths of 10, 30, and 50 cm in solarized and control plots.

T. harzianum preparation (60 g/2 m²/tree) was incorporated into the soil of existing nonsolarized trees in July 1984 and to solarized ones after tarp removal.

Efficacy of treatments for control of _R. necatrix_ was evaluated with three tests:

1. Naturally infected root segments (1.5–2.0 cm long) were buried in groups of 20 in each plot in the soil at various depths ranging from 10 to 60 cm in the tarped and control sites and at a 10-cm depth in soil in the _T. harzianum_ plot before solarization was started; 2, 4, and 8 wk later, the segments were removed from the soil and incubated in moisture chambers for 1 wk to determine mortality of _R. necatrix_. The segments were in groups of 20 in nylon net bags attached to a nylon cord buried in the soil and were lifted without significantly affecting soil temperature. Control efficiency was expressed as percent mortality of the pathogen.

2. Soil samples taken from solar and nonsolar treatments, immediately after and at 280 days after solarization had been terminated, were assessed for the presence of _R. necatrix_ by the leaf colonization method.

3. Two weeks after solarization had been terminated, 6-mo-old avocado plants were planted in the location of the uprooted trees to evaluate disease incidence. Each treatment consisted of 25 plants, with five samples per plot. Avocado plants in the two _T. harzianum_ treatments were each drenched with a 50-ml _T. harzianum_ spore suspension (10^8/ml) at planting time. Disease incidence was determined by assessing plant mortality over a period of 10 mo after solarization.

Solarization in existing orchard. Before commencing solarization and 14 and 25 mo later, the trees were rated with the following disease index: 0 = healthy with full canopy of foliage, 1 = mild chlorosis and a few dry branches, 2 = considerable chlorosis and many dry branches, and 3 = dead tree.

Induced suppressiveness to _R. necatrix_. In the first approach, naturally infested En Zurim soil was mixed (1:1, w/w) with noninfested adjacent soil or with adjacent solarized soil. The inoculum level of _R. necatrix_ in these soils, after various incubation periods, was determined by the avocado leaf colonization method. In the second approach (14), quantities of 25 g of solarized and nonsolarized soils were placed in plates (9 cm diameter). Boiled cellophane membranes with four MEA culture disks (4 mm diameter) of _R. necatrix_ were laid on the soil surface and incubated at 25 C for 24 hr. The mycelial growth on the disks was assessed microscopically. The length of five hyphal threads was measured in each of four microscopic fields per disk. Mycelial growth index was evaluated as
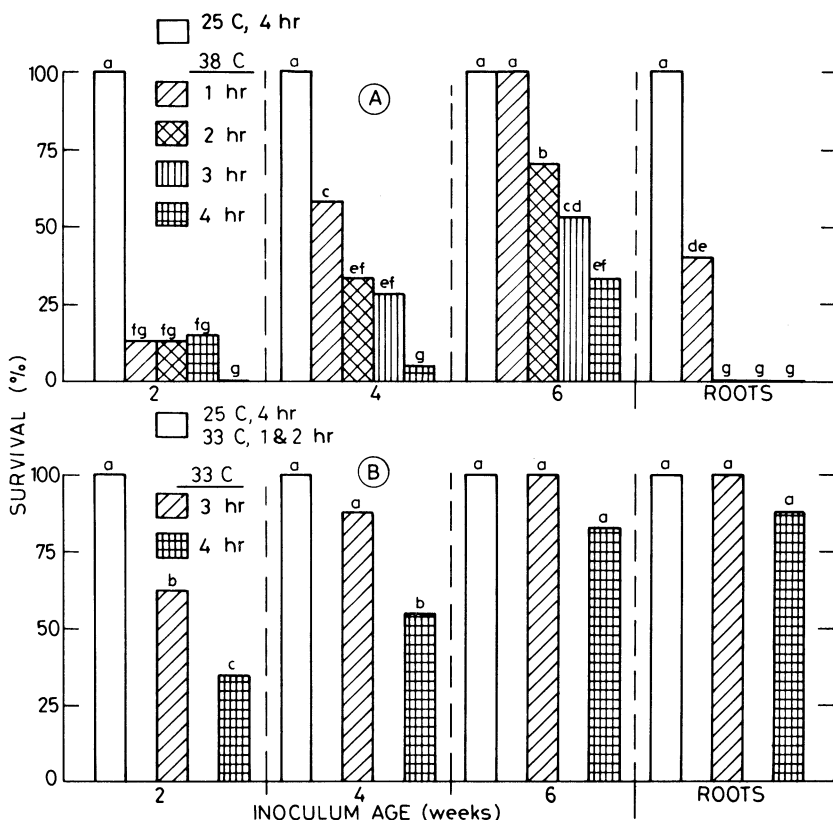

Fig. 2. Heat sensitivity of _Rosellinia necatrix_ at two temperatures (**A and B**) in inoculated wheat seeds as affected by inoculum age. For comparison, naturally infected apple roots were subjected to the same heat treatments. Values at each temperature with a common letter are not significantly different ($P = 0.05$).

average relative length of hyphae × number of hyphae within a microscopic field at 10 × 15 magnification.

Statistical analysis of the data was determined by Duncan's multiple range test, factorial analysis, or linear regression analysis as indicated, with a significance level of $P = 0.05$.

RESULTS

Heat sensitivity of inocula of *R. necatrix*. Viability of the pathogen, expressed as percentage of avocado leaf colonization in a soil artificially infested with inoculated wheat seeds, was inversely related to increasing temperatures (from 34 to 38 C) or exposure time (Fig. 1). Inoculum in inoculated wheat seeds was much more sensitive to heat treatment than artificially infested soil. Heat sensitivity of *R. necatrix* was affected by inoculum quality because sensitivity decreased with aging of artificially inoculated wheat seeds (Fig. 2A,B). In comparison, naturally infected roots were the least heat-sensitive at 33 C and the most sensitive at 38 C.

Effects of combined physical and biological treatments on *R. necatrix* viability. Heating naturally infested En Zurim soil or treating it with *T. harzianum* reduced leaf colonization (Fig. 3). Combining the treatments further reduced colonization. These treatments were less effective for control of the pathogen in another naturally infested soil (Hanita), where the natural inoculum was at a higher density and was less heat-sensitive. Sensitivity of the inocula in these two naturally infested soils to heat treatment was higher than that of artificially infested soil (Fig. 1).

Pathogen and disease control by soil solarization and *T. harzianum*. Maximum soil temperatures are presented in Table 1. Temperatures were highest in the solarized plots and decreased with soil depth. Tarping the soil in solarized plots increased soil temperatures by 5–11 C over the respective nonsolarized controls. Soil temperatures in the tarped-shaded plots were only slightly higher than those in the nonsolarized plots. Soil temperatures at 60 cm deep were not recorded, but the calculated maximum temperatures using suitable equations (25) were 29.5, 35.2, and 31.1 C for nonsolarized, solarized, and tarped-shaded plots, respectively. Various approaches were followed to determine solarization efficacy for control of *R. necatrix*. Mortality rate of the surviving pathogen population in segments of naturally infected roots buried in the soil of the various plots was determined periodically after tarping. Results (Fig. 4) show that the pathogen remained viable in the untreated and *T. harzianum*-treated plots at all tested depths throughout the 8-wk trial period. Solarization resulted in a decline of pathogen recovery that progressed with time and was most

pronounced at the upper soil layers. After 28 days of solarization, the pathogen was completely eradicated at soil depths of 10–30 cm. After 56 days of solarization, 75% pathogen mortality was recorded at 60 cm deep. Pathogen mortality in the tarped-shaded plots was less pronounced, resulting in kill percentages after 56 days of 38 and 12% at depths of 10 and 30–60 cm, respectively.

The effect of solarization on pathogen population in naturally infested soil was determined by taking soil samples from depths of 10 and 30 cm 56 days after tarping. The relative pathogen population was estimated with the avocado leaf colonization method. Colonization percentages in the untreated soil at depths of 10 and 30 cm were 58.3 and 33.3, respectively. Colonization declined to zero in both previously solarized and tarped-shaded plots at 10 and 30 cm deep. Soil samples were also taken from the field 280 days after removal of the polyethylene sheets. Colonization percentages were 35 and 13.3% in the untreated plots at 10- and 30-cm depths, respectively. The percentage of *R. necatrix* colonization of soil from the solarized plots at 10- and 30-cm depths

remained zero.

The effectiveness of solarization and *T. harzianum* treatments for disease control in the naturally infested soil was also examined by planting avocado plants in this soil. Diseased plants in the untreated plots were first detected in April 1985, 7 mo after termination of the solarization treatment (Fig. 5). By the end of the experiment, 3 mo later, disease percentages in the control and *T. harzianum* plots were 52 and 40%, respectively. Both solarization treatments were completely effective in controlling the disease throughout the experimental period.

The effectiveness of solarization for control of the disease in existing trees in the orchard was examined on a limited scale. Results (Table 2) show that the two solarized treatments reduced the disease to negligible levels over 25 mo at least, whereas disease levels of trees in the untreated plots or treated with *T. harzianum* increased during the 2 yr of the experiment. No apparent damage was observed in trees subjected to solarization.

Behavior of *R. necatrix* in solarized soils. Two approaches were followed for determining the fate of the pathogen
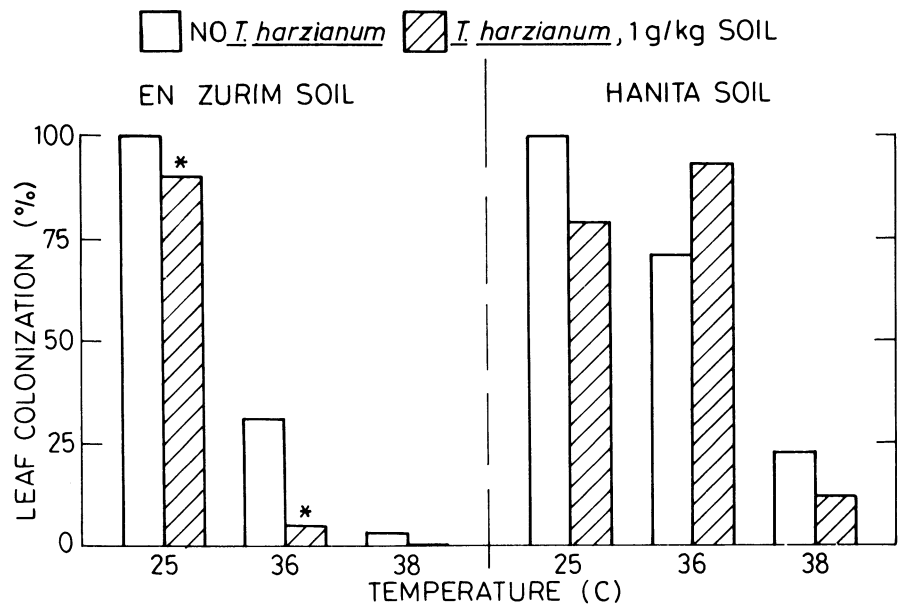

Fig. 3. Effects of heating for 4 hr and *Trichoderma harzianum*, separately or combined with heating, in two naturally infested soils, on *Rosellinia necatrix* survival, assessed by the leaf colonization method. In both soils, following a factorial analysis, a significant interaction ($P = 0.05$) existed between *T. harzianum* and temperature. A significant difference existed between all temperatures in the no *T. harzianum* treatments ($P = 0.05$). An asterisk denotes a significant effect ($P = 0.05$) of *T. harzianum* at the respective temperatures.

Table 1. Maximum soil temperatures recorded in a solarized apple orchard during July 1984 at En Zurim, Israel

Soil depth (cm)	Maximum soil temperature (C)		
	Nonsolarized	Solarized	Solarized, shaded
10	35	46	37
30	33	38	33
50	31	37	32

introduced into previously solarized or untreated soils. Naturally infested soil served as the inoculum source and was mixed with either naturally noninfested field soil or with the comparable solarized soil, and subsequently, the colonization percentage of avocado leaf disks by the pathogen was determined. Results (Fig. 6) show that the solarized soil suppressed pathogen activity, especially after an extended period of incubation. After 28 days of incubation, colonization in the solarized soil mixture declined to 7% compared with 28% in the nonsolarized soil mixture.

Agar culture disks of the pathogen were laid on either nonsolarized or solarized soil, and growth rate of the pathogen was determined after 24 hr of incubation. Results from two of six typical experiments (Table 3) show a similar trend, namely, that the solarized soil suppresses pathogen growth, thus confirming the results with the same soils by the colonization approach (Fig. 6). Samples of solarized soil taken from the field 9 mo after solarization and tested by the mycelial growth method remained suppressive to *R. necatrix.*

DISCUSSION

Soil solarization is very effective in controlling white root rot disease and in reducing the population or activity of *R. necatrix* in soil to the depth of at least 60 cm. This was shown by using various approaches for assessing levels of inocula of various types. The successful control of *R. necatrix* by solarization that lasted for at least 25 mo in certain cases might be attributed to a variety of physical, chemical, and biological mechanisms. *R. necatrix* is highly sensitive to heat, as shown in other studies (3), more so than *Verticillium dahliae,* which is a heat-sensitive fungus (18). The reduced heat sensitivity of the pathogen after aging may be due to the formation of sclerotia and other melanin-containing structures (22). In the solarized soil, activity and growth of the pathogen were suppressed (Table 3, Fig. 6), indicating the existence of biological control processes. The pattern of mycelial growth suppression in the solarized soil (Table 3) that was evident for at least 9 mo is similar to that observed with *Phytophthora cinnamomi* in solarized soil (17) and with *Rhizoctonia solani* in suppressive soils (14). Similarly, incidence of diseases caused by *Sclerotium rolfsii, Fusarium oxysporum* f. sp. *lycopersici,* and *F. oxysporum* f. sp. *dianthi* was lower in the solarized soil because of induced suppressiveness and enhanced antagonistic activity (7,9). Thus, it appears that biological control in solarized soils is not an exceptional phenomenon. Inoculum of *R. necatrix* in a naturally infested soil was eradicated to a depth of 30 cm in a tarped-shaded soil where soil temperatures were only slightly higher than those in the untarped soil. This might be due to the accumulation of volatiles under the polyethylene tarp (10). In a similar study, various nematode populations decreased in moist poly-ethylene-covered but shaded soil (20). A weakening effect by sublethal temper-atures, which further facilitates biological control as shown with *Armillaria mellea* (16) and *S. rolfsii* (15), may also operate in soil during solarization. Thus, a combination of mechanisms is involved in the drastic reduction of inoculum in the solarized soil, finally leading to a long-term effect of disease control, as described (11).

T. harzianum was not effective in the present study when applied in the field. This could be attributed, among other things, to an inadequate mode of
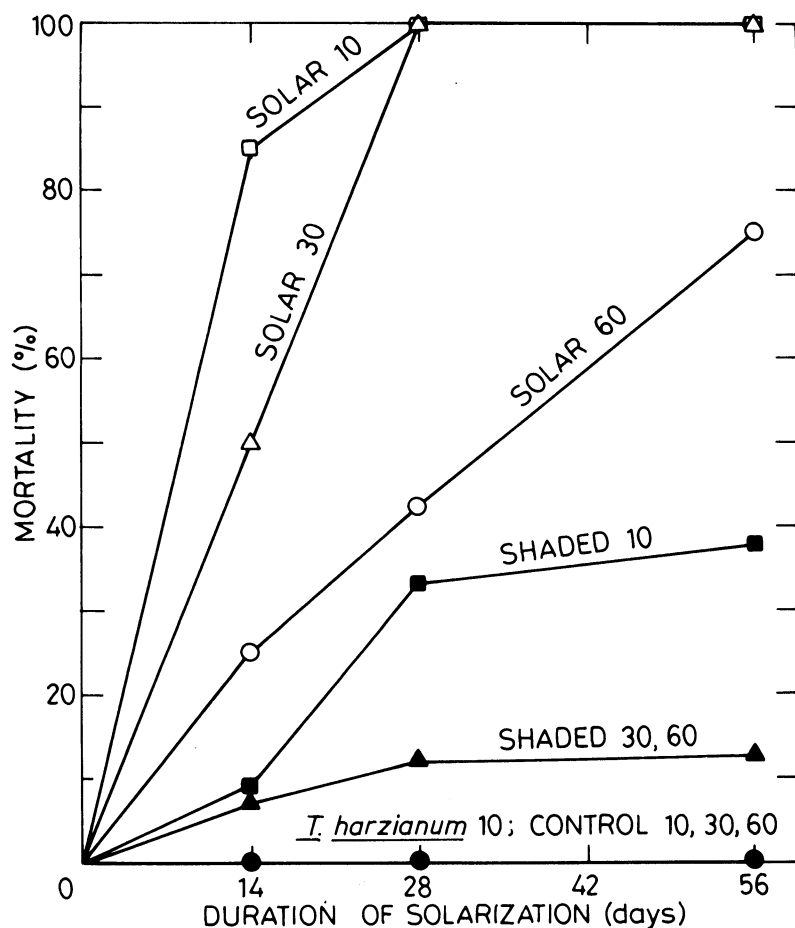

Fig. 4. Effects of solarization and *Trichoderma harzianum* on the mortality of *Rosellinia necatrix* in an apple orchard. The inoculum, consisting of naturally infected apple root segments (1.5–2 cm), was buried in the soil at three depths, removed after various periods, and tested for viability. Numbers on graph lines indicate soil depth (cm). Solar = solarized sites far from the shaded area; shaded = solarized sites under the tree canopy; *T. harzianum* = sites treated by *T. harzianum* at 1 g of preparation per kilogram of soil; and control = untreated sites. Mortality percentage remained zero throughout the test period in *T. harzianum*-treated and control soils at the tested depths. Following ln transformation of *X* coordinate, the linear regression coefficients (0.95 < *r* < 0.98) are significant except for graph line indicating shaded 10. All slopes are significantly different from zero (graph line indicating control and *T. harzianum* treatments) (*P* = 0.05).
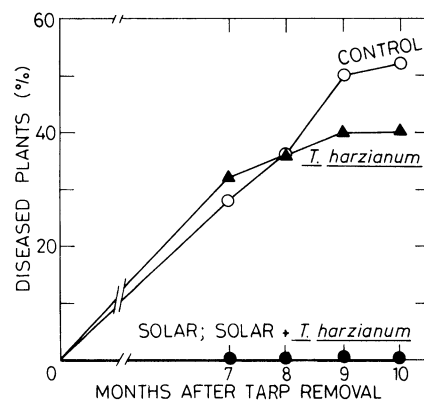

Fig. 5. Effectiveness of solarization and *Trichoderma harzianum* for control of *Rosellinia necatrix* in a naturally infested soil. Soil was tarped during July and August 1984. Avocado plants were planted in October 1984 in the tested soil. Percentage of plants with white root rot symptoms was assessed at various times thereafter. Solar = plants in solarized sites; *T. harzianum* = plants treated with 50 ml of *T. harzianum* spore suspension (10^8 conidia per milliliter); and control = plants in untreated sites. Following logit [(ln $x/(100 - x)$] transformation of *Y* coordinate, the linear regression coefficients (0.94 < *r* < 0.97) are significant. Slopes of the graph lines indicating nonsolar treatments are significantly different from the slope of graph line indicating solar treatments (*P* = 0.05).

Table 2. Effects of soil solarization and *Trichoderma harzianum* in an existing orchard on white root rot disease in apple trees[w]

Treatment	Disease index[x]			
	July 1984	September 1985	August 1986	Change[y] (%)
Untreated	1.0 a[z]	2.0 a	2.50 a	+150
T. harzianum	1.2 a	2.2 a	3.00 a	+150
Solarization	1.2 a	0.2 b	0.04 b	−97
Solarization + *T. harzianum*	0.4 a	0.0 b	0.00 b	−100

[w] Disease rating was carried out before tarping in July 1984, September 1985, and August 1986.

[x] Disease was rated on a scale where 0 = healthy and 3 = dead tree.

[y] Percent change in disease incidence from July 1984 to August 1986, calculated as $[(A - B/A)] \times 100$.

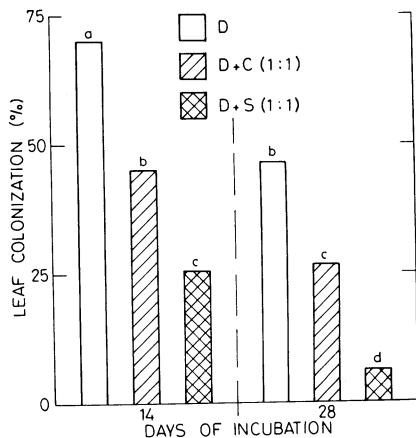[z] Values in each year having a common letter are not significantly different ($P = 0.05$).

Fig. 6. Effect of solarized soil on colonization capacity of *Rosellinia necatrix*. Naturally infested soil (D) served as an inoculum source and was mixed with either noninfested soil or the comparably solarized soil. The soils were incubated at 25 C for 14 or 28 days before leaf colonization assessment. Values with a common letter are not significantly different ($P = 0.05$). C = noninfested soil; S = solarized soil.

Table 3. Mycelial growth index[y] of *Rosellinia necatrix* in solarized and nonsolarized En Zurim soil

Soil treatment	Exp. 1	Exp. 2
Untreated	118.2 a[z]	140.0 A
Solarized	39.6 b	56.0 B
Reduction (%)	66.5	60.0

[y] Evaluated as average relative length of hyphae × number of hyphae within a microscopic field at 10 × 15 magnification.

[z] Values with a common letter are not significantly different ($P = 0.05$).

application. Various studies have shown that combining solarization with other methods of control, e.g., *T. harzianum* (2,4), metam-sodium (Vapam [5]), or crop rotation (11), improved pathogen control or extended it. Combining partial heating with reduced dosage of *T. harzianum* resulted in an improved control of the pathogen in En Zurim soil (Fig. 3). The possibility that such a combination could lead to a more lasting control should be studied further.

Soil solarization was effective in controlling *R. necatrix* in an existing orchard, as was also found for *V. dahliae* in pistachio (1) and olive groves (24). In the present case, soil solarization fulfills the requirements for successful control of a soilborne pathogen in an existing orchard (1,10): The tree was not damaged, the inoculum was controlled to a considerable depth, soil reinfestation was delayed, and in the tarped-shaded area, the inoculum was also reduced. Soil solarization is a promising method of soil disinfestation that can be applied as a postplanting treatment. The apple rootstock survived the high temperatures prevailing during solarization even though it is considered heat-sensitive (8). This may be due to a higher heat tolerance of this rootstock. Certain chemical treatments that reduce heat damage (8) should be considered where necessary. Compared with annual crops, the control of soilborne pathogens in existing orchards presents difficulties because the pathogen has to be controlled to greater depths and for longer periods of time without damage to trees. Therefore, integrated methods of control should be given a high priority in such future research programs.

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