

# BARD

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**FINAL REPORT**

**PROJECT NO. IS-1291-87**

## **Postplant Control of Soilborne Diseases of Fruit Tree Crops by Soil Solarization**

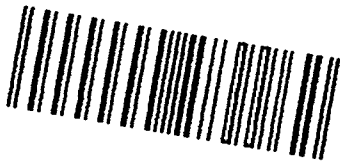
**J. Katan, J.E. Devay, E. Shabi, Y. Pinkas**

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**Postplant control of soilborne diseases of fruit tree crops by soil solarization**

Investigators' Names

Investigators' Institutions

J. Katan

The Hebrew University

J.E. DeVay

University of California, Davis

E. Shabi

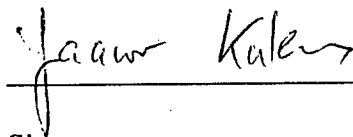
ARO, The Volcani Center

Y. Pinkas

ARO, The Volcani Center

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Signature

Principal Investigator



Signature

Institute's Authorizing Official

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632.3 : 634

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### ABSTRACT

This project deals with the effect of postplant solarization, especially with fruit tress, on pathogen and disease control, and on physical, chemical and biological changes on the solarized plant.

In Israel, solarization of an apple orchard effectively controlled population of Rosellinia necatrix and the white rot disease. Disease control lasted for three years with a full recovery of the trees and reduction in disease in apple. Studies were also carried out with other diseases including Verticillium in almonds, Phytophthora cinnamomi in avocado and replant diseases in peaches and apples. Solarization of apple plants adversely affected photosynthesis and respiration and caused chlorosis, but the plants recovered later and their growth was improved in the following two years. Solarization stimulated beneficial fluorescent pseudomonades bacteria. Combining solarization with organic and inorganic amendments improved pathogen control.

In the USA, the incidence of foliar wilt symptoms and vascular discoloration due to Verticillium wilt disease were reduced by 86% to 100% in both apricot and almond trees by both clear and black films. Trees solarized with clear film did not survive or grow as well as those solarized with black film or the nonsolarized. The intermediate soil temperatures under black films did not injure the roots, while the prolonged period of soil heating under black film provided control of Verticillium wilt equivalent to that obtained with clear film. Significant conservation of irrigation water was achieved by postplant solarization. In another study, the heat sensitivity and the effect of soil solarization on the survival of hyphae and spores of three major soilborne pathogens, Phytophthora cinnamomi, P. cactorum, and P. megasperma were determined. The heat sensitivity in laboratory experiments closely reflected their inactivation in solarized soil. No viable spores of P. cinnamomi were detected in infested soil after solarization for 2 wk, at 30 cm depth. P. cactorum was killed within 2 wk at the 15 cm depth but withstood the effects of soil solarization at the 30 to 45 cm depths. P. megasperma was the least heat sensitive of the isolates used, but soil solarization for 4 wk greatly reduced the number of its viable propagules. The results of these studies demonstrate the potential of postplant solarization.

### Objectives of the research

1. To study the effectiveness of solarization (alone or combined with biocontrol agents) in the control of soilborne pathogens in existing orchards, as a postplant treatment.
2. To study the effect of solarization on the roots of the solarized trees as affected by the heat, the conservation of soil moisture, and by gas changes in the soil.
3. Mechanisms of solarization in existing orchards: to study reinfestation by pathogens, gases (e.g., ethylene) accumulating in soil, and the temperature regime in the solarized orchard with various degrees of shading.

### Description of the research

#### Introduction

This project deals with postplant solarization of fruit trees, which differs in many aspects from the conventional preplant solarization for disease control in annual crops. In both cases, hydrothermal processes are generated in the soil by plastic tarping, thus increasing soil temperatures and enhancing biological processes, and if conditions are optimal, finally leading to pathogen and disease control as well as yield increase. While in preplanting solarization these processes take place in the absence of the host plant, postplanted solarization is carried out in the presence of the host. Therefore, adverse effects on the plant, due to its exposure to high temperatures during solarization are possible; they should be studied and avoided.

In general, the present project deals with the following major topics:

- a. The effectiveness of postplant soil solarization in reducing pathogen population and in controlling soilborne diseases, in a variety of fruit trees.
- b. Effect of postplant solarization on physical, chemical and biological processes in the soil, e.g. soil temperature, soil moisture, and change in populations of beneficial and harmful soil microorganisms.

Since this project deals with trees, efforts have been made to also study the long term effects of solarization, for more than one growing season. For this purpose, we also used for our studies orchards which have been solarized in



the previous years. The studies that were carried out in Israel and in the USA have dealt with fruit trees from different families, and of various ages young trees, mature trees and replanted trees.

# I - STUDIES IN ISRAEL

## 1. Long-term effect of postplant soil solarization for the control of *Rosellinia necatrix* in apple

*Rosellinia necatrix* Prill. (anamorph: *Dematophora necatrix* Hartig) is the cause of white root rot disease in many fruit trees, including apple. The pathogen causes root rot, followed by leaf chlorosis, premature leaf fall, wilting and finally, death of the tree.

The control of soil-borne pathogens poses special difficulties with perennial crops, as any preplanting treatment needs to have a long-term effect and ant post-planting treatment must not adversely affect the crop plant (Katan, 1987). Various approaches are possible for the control of *R. necatrix* before planting, for instance soil fumigation using methyl bromide, soil solarization, or biological control using the antagonist *Trichoderma harzianum*. Solarization has been used successfully for the control of *R. necatrix* as a preplanting treatment for soil disinfestation and as a postplanting curative treatment for diseased tree (Sztjenberg et al, 1987).

In the study reported here, we examined the long-term effect of soil solarization, both as preplanting and postplanting treatments over 2-4 year period, for the control of *R. necatrix* in naturally infested soil and in an existing apple orchard. Parts of this work on long-lasting control of *R. necatrix* in an existing orchard are a continuation of a previous study (Sztjenberg et al, 1987). (List of the references is given in the enclosed article).

## Summary of the results

The effect of pre- and post-planting soil solarization on white root rot of apple caused by *Rosellinia necatrix* were examined in two naturally infested orchards. The pathogen was eradicated in solarized soil to a depth of 30 cm. Partial or complete destruction of the pathogen was obtained in solarized-shaded (partially shaded by the tree canopy)

plots. No reinfestation of solarized and solarized-shaded soil was observed two years after treatment. No death of replanted apple tree occurred in the solarized plots up to two years after solarization, whereas 60% of tree died in untreated plots. No disease developed during the third year after solarization in an existing apple orchard, but a low rate of disease reoccurred in the fourth year. In contrast, mortality in diseased trees in the absence of solarization reached 100% 4 years after treatment. Two previously solarized soils, out of seven tested, caused reduced growth of the pathogen, indicating induction of soil suppressiveness by solarization. Further details are given in the enclosed article.

The effectiveness of solarization in controlling R. necatrix in an existing apple orchard under commercial conditions, was examined. A 6-years old apple orchard in the northern part of Israel, showed typical symptoms of the white rot disease. In the central part of the orchard, 30% disease was recorded. The involvement of R. necatrix in the disease was verified by plating root segments on potato dextrose agar. The whole orchard (4000 m<sup>2</sup>) was solarized in summer 1990, as described above (Freeman et al, 1990). The effect of solarization on the control of the pathogen was assessed by incorporating infected root segments at 10 and 30 cm depth in the solarized plot and in a nonsolarized adjacent plot. After the termination of solarization, viability tests of pathogen showed that solarization completely controlled the pathogen at the two tested depths. No adverse effects on the solarized (heated) trees were evident.

No disease symptoms were found during the following year. Apparently, a full recovery of the trees was achieved.

## 2. Solarization for the control of Sclerotium rolfsii in apple:

The soil fungus Sclerotium rolfsii is a pathogen of many field crops as well as of apple. The optimal temperature for pathogen growth is 30-32 C. It has been suggested in the past to reduce the incidence of this disease by mulching the soil with straw in order to reduce soil temperatures to levels which are suboptimal to the pathogen. However, this issue is controversial.

## Long-term effect of soil solarization for the control of *Rosellinia necatrix* in apple

S. FREEMAN, A. SZTEJNBERG, E. SHABI\* AND J. KATAN

Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel and \*Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

**ABSTRACT.** The effects of pre- and post-planting soil solarization on white root rot of apple caused by *Rosellinia necatrix* were examined in two naturally infested orchards. The pathogen was eradicated in solarized soil to a depth of 30 cm. Partial or complete destruction of the pathogen was obtained in solarized-shaded (partially shaded by the tree canopy) plots. No reinfestation of solarized and solarized-shaded soil was observed 2 years after treatment. No death of replanted apple trees occurred in the solarized plots up to 2 years after solarization, whereas 60% of trees died in untreated plots. No disease developed during the third year after solarization in an existing apple orchard, but a low rate of disease recurred in the fourth year. In contrast, mortality in diseased trees in the absence of solarization reached 100%, 4 years after treatment. Two previously solarized soils, out of seven tested, caused reduced growth of the pathogen, indicating induction of soil suppressiveness by solarization.

**KEYWORDS:** Apple; *Dematophora necatrix*; *Rosellinia necatrix*; solar heating; solarization; white root rot

### Introduction

*Rosellinia necatrix* Prill. (anamorph: *Dematophora necatrix* Hartig) is the cause of white root rot disease in many fruit trees, including apple (Sztejnberg and Madar, 1980). The pathogen causes root rot, followed by leaf chlorosis, premature leaf fall, wilting and finally, death of the tree.

The control of soil-borne pathogens poses special difficulties with perennial crops, as any preplanting treatment needs to have a long-term effect and any post-planting treatment must not adversely affect the crop plant (Katan, 1987; Shabi, Pinkas and Katan, 1987; Sztejnberg *et al.*, 1987). Various approaches are possible for the control of *R. necatrix* before planting, for instance soil fumigation using methyl bromide (Sztejnberg, Omary and Pinkas, 1983), soil solarization (Sztejnberg *et al.*, 1987), or biological control using the antagonist *Trichoderma harzianum* (Freeman, Sztejnberg and Chet, 1986). Solarization has been used successfully for the control of *R. necatrix* as a preplanting treatment for soil disinfestation and as a post-planting curative treatment for diseased trees (Sztejnberg *et al.*, 1987).

In the study reported here, we examined the long-term effect of soil solarization, both as preplanting and post-planting treatments over a 2–4-year period, for the control of *R. necatrix* in naturally infested soil and in an existing apple orchard. Parts of this work on long-lasting control of *R. necatrix* in an existing orchard are a continuation of a previous study (Sztejnberg *et al.*, 1987).

### Materials and methods

#### *Isolates of R. necatrix*

Isolates of *R. necatrix* were obtained from roots of naturally diseased apple (*Malus sylvestris* Mill.) trees of various cultivars from two orchards, at En Zurim in the coastal plain and at Massade in the Golan heights.

#### *Control of R. necatrix under field conditions*

Field experiments were carried out in naturally infested soil in a 4-year-old apple orchard at Massade (800 m altitude) and in a 15-year-old apple orchard at En Zurim (50 m altitude). The field trial in an existing orchard was set up at En Zurim in 1984 and initial results have previously been described and reported (Sztejnberg *et al.*, 1987).

#### *Solarization trials in naturally infested soil of Massade*

Eight plots (6 × 4 m) were selected randomly: four were solarized and the other four were non-solarized controls. Each plot contained the area of one tree that had been killed by *R. necatrix* and removed before the experiment started. Trees in the orchard were spaced 4 m apart and the area in the solarized plots was adjacent to an existing tree. Approximately 1 m<sup>2</sup> of the solarized area in the vicinity of the adjacent tree, not exposed to direct sunlight and shaded during most of the day, is referred to as the solarized-shaded site. Soil to be solarized was pre-irrigated by a sprinkler system 48 h before treatment. Solarization was carried out on

15 July 1986 by covering the soil with transparent polyethylene sheeting 40 µm thick and was terminated 8 weeks later. During the trial period, soil temperatures in Massade were recorded with a thermometer inserted in the soil at depths of 10 and 30 cm, at 1600–1900 h.

Efficacy of treatments for control of the pathogen was evaluated by three tests, as follows.

For the first test, naturally diseased root segments (1.5–2.0 cm long) from the orchard of Massade were buried in groups of five in the soil, in the centre of each plot at a depth of 30 cm in the solarized, solarized-shaded and non-solarized control sites, before solarization was started. After solarization, root segments were removed from the soil and incubated in moisture chambers for 1 week at 25°C to determine viability of the pathogen by monitoring mycelial growth from the roots.

For the second test, soil samples taken from non-solarized and solarized-shaded sites before solarization and from solarized, solarized-shaded and non-solarized plots 7 days after solarization (referred to as immediately after solarization) and 2 years later, were assessed for the presence of *R. necatrix* by the avocado leaf colonization method (Sztejnberg *et al.*, 1987). Each soil sample was moistened to field capacity and placed in a plastic container (11 × 11 × 4 cm) holding 250 g soil. Avocado leaf discs (1.6 cm diameter) were buried in the soil to serve as traps for the pathogen. The containers were incubated in light at 25°C for 12–14 days, after which leaf colonization was assessed. Discs colonized by *R. necatrix* developed characteristic white mycelium and underwent a colour change to cream or light brown, whereas uncolonized discs remained green. Four soil samples were taken from each plot on each sampling occasion and each sample was baited with 15 leaf discs. Results were expressed as percentage of disc colonization.

For the third test, 3 months after solarization had been terminated, six 1-year-old apple trees (cv. Granny Smith, rootstock M 109) were planted in a row 50 cm apart, in each of the solarized and non-solarized untreated plots. Disease incidence was determined by assessing the percentage of dead trees up to 2 years after solarization. The roots of the dead trees were examined microscopically to confirm the presence of *R. necatrix*.

#### *Long-term results of solarization in an existing orchard of En Zurim*

In an orchard showing initial disease symptoms, 20 plots (10 × 6 m) containing one established tree each were selected at random and half were solarized. Solarization was carried out at the beginning of July 1984 by covering the sprinkler pre-irrigated soil with transparent polyethylene sheeting 40 µm thick. The sheet was removed 8 weeks later. The solar-treated trees were situated in the central area of the polyethylene sheeting. In each treatment, five trees were also

treated with a *T. harzianum* preparation as previously described (Sztejnberg *et al.*, 1987). There was no effect of *T. harzianum* on disease control; the results of the *T. harzianum* treated and untreated plots were therefore pooled. Before solarization, and annually over a 4-year period, the trees were scored visually on a disease index: 0, healthy with full canopy of foliage; 1, mild chlorosis and a few dead branches; 2, considerable chlorosis and many dead branches; 3, dead tree. The average disease index for each treatment before solarization is given in Figure 4. More experimental details are given elsewhere (Sztejnberg *et al.*, 1987).

#### *Induced suppressiveness of R. necatrix*

The method used was essentially that described previously (Sztejnberg *et al.*, 1987). Solarized and non-solarized soils of various types were collected from different locations in Israel, including soil from the experimental site in the apple orchard of En Zurim. Quantities (25 g) of solarized and non-solarized soils were moistened to field capacity and placed in Petri dishes (9 cm diameter). The soil surface was covered with boiled cellophane membranes and four malt-extract agar culture discs (4 mm diameter) of *R. necatrix* were laid on the soil surface and incubated at 25°C for 24 h. The extent of mycelial growth from the discs was assessed microscopically. The lengths (µm) of five hyphae threads were measured in each of four microscopic fields per disc.

Statistical analyses of the data were determined by factorial analysis or Duncan's multiple range test, as indicated, with a significance level of  $p = 0.05$ . Data in percentages were transformed to corresponding arcsine values before analysis.

## Results

#### *Effect of soil solarization on inoculum of R. necatrix*

Maximal soil temperatures recorded in the apple orchard of Massade during July 1986 at 10 cm depth were 34°C in the non-solarized plots, 37.5°C in the solarized-shaded plots and 44°C in the solarized plots. The corresponding temperatures at 30 cm depth were 32, 35 and 39°C, respectively.

The pathogen population surviving in root segments was completely eradicated in the solarized plots, compared with a partial mortality of 40% in the solarized-shaded plots (Figure 1). The pathogen remained 100% viable in the untreated non-solarized plots.

The effect of solarization on the pathogen population in naturally infested soil at the depth of 30 cm was determined before and immediately after solarization, using the leaf colonization method (Figure 2). Before solarization, colonization percentages in the open and shaded plots were 63 and 69%, respectively. Immediately after solarization, colonization in soil from the treated plots (solarized and solarized-shaded) was zero, whereas that in the untreated plots was 41%.

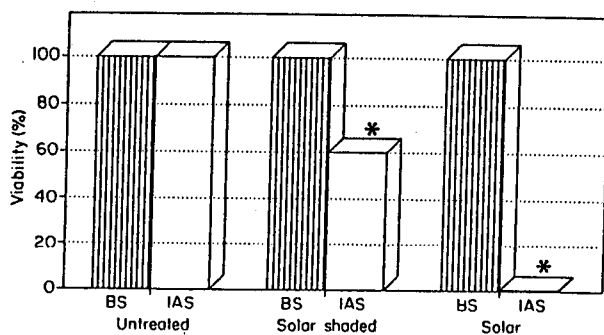


FIGURE 1. Effect of solarization on viability of *Rosellinia necatrix* in naturally infested apple root segments in the orchard soil. The segments were tested for viability before solarization (BS) and 7 days after solarization (IAS). Factorial analysis of the data indicated a significant interaction ( $p=0.05$ ) between treatments and period of viability testing. Differences between viability of IAS in all treatments were significantly different ( $p=0.05$ ). \*Significant difference ( $p=0.05$ ) in viability between BS and IAS at the respective treatments

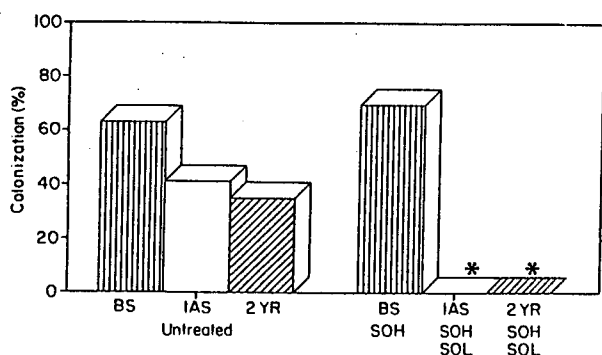


FIGURE 2. Effect of solarization on survival of *Rosellinia necatrix* in naturally infested soil, assessed by avocado leaf colonization. Percentage colonization was assessed before solarization (BS), 7 days after solarization (IAS) and 2 years later (2 YR). SOH, solarized-shaded; SOL, solarized. Factorial analysis of the data indicated a significant interaction ( $p=0.05$ ) between treatments and period of colonization testing. Percentage colonization of the BS period was significantly different ( $p=0.05$ ) from the IAS and 2 YR periods in both the untreated and solarized treatments. \*Significant difference ( $p=0.05$ ) for the respective treatments in the SOH and SOL treatment

#### Long-term effect of soil solarization

The potential long-term effect of solarization for the control of *R. necatrix* was examined by various approaches. Two years after solarization, pathogen population levels in soil, as assessed by the leaf colonization method, was 35% in the untreated control and declined to zero in both solarized and solarized-shaded plots (Figure 2).

The effectiveness of solarization for disease control in naturally infested soil was also examined by planting apple trees in solarized and untreated soil (Figure 3). One year after solarization, the mortality of trees in the untreated plots reached 30%, whereas no trees died in the solarized plots. Two years after solarization, the incidence of tree death had further increased to 60% in the untreated plots, while in the solarized plots all the plants remained healthy.

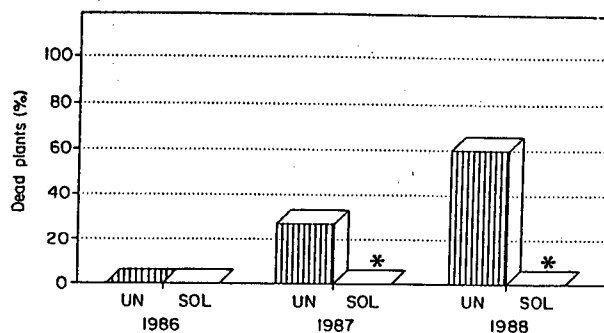


FIGURE 3. Incidence of apple tree death due to *Rosellinia necatrix* in soil with or without solarization treatment. Soil was solarized from July 1986 for a period of 8 weeks. One-year-old apple trees were planted in the untreated (UN) and solarized (SOL) plots, 3 months after solarization. Data are percentages of plants killed by the pathogen, 1 and 2 years after solarization. Factorial analysis of the data indicated a significant interaction ( $p=0.05$ ) between each year and treatments. Differences between the untreated plants in all years were significant ( $p=0.05$ ). \*Significant effect ( $p=0.05$ ) of solarization at the respective years

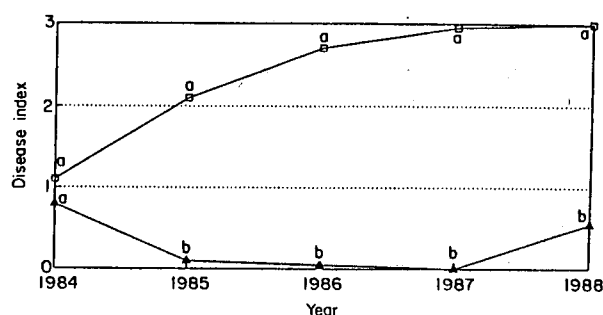


FIGURE 4. Effect of solarization as a post-planting treatment for the control of *Rosellinia necatrix* in an apple orchard at En Zurim. Trees of the solar (lower curve) and untreated (upper curve) plots were rated immediately before solarization in 1984 and for four consecutive years with a disease index on the scale: 0, healthy; 3, dead tree. Values with a common letter in each year are not significantly different ( $p=0.05$ )

The reduction in disease incidence in an existing orchard at En Zurim during the first 2 years after solarization (1984–86) has been reported (Sztejnberg *et al.*, 1987); these data are repeated here for comparison (Figure 4). Observations in 1987 show a further increase in disease index in untreated trees, which was equivalent to 98% of the maximum, whereas disease index declined to zero in the solar-treated trees. By October 1988, all untreated trees had died, and some disease symptoms in trees in the solarized plots were observed, although the average disease index did not exceed that recorded before treatment (1984) in the solarized trees.

#### Induced suppressiveness of *R. necatrix*

Agar culture discs of the pathogen were laid on either non-solarized or solarized soils, and growth rate was determined. The solarized soil from En Zurim (naturally infested with *R. necatrix*) and Mahanayim, significantly suppressed growth of the pathogen (Figure 5; Table 1), whereas solarized soils from Bet Hashitta and

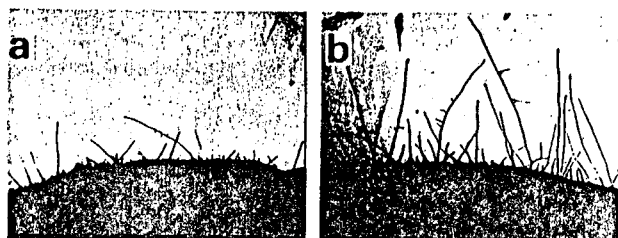


FIGURE 5. Growth of *Rosellinia necatrix* from culture discs on cellophane over (a) solarized and (b) non-solarized soil from En Zurim

TABLE 1. Mycelial growth\* of *Rosellinia necatrix* in solarized and non-solarized soils from different locations in Israel

Location	Soil type	Mean hyphal length ( $\mu\text{m}$ )	
		Non-solarized	Solarized
En Zurim <sup>b</sup>	Vertisol	281 a <sup>c</sup>	115 b
Bet Hashitta	Vertisol	297 a	287 a
Mahanayim	Peat soil	329 a	190 b
Sede Eliyyahu	Rendzina	297 a	299 a

\*Average length ( $\mu\text{m}$ ) of hyphae within a microscopic field at magnification  $\times 150$ ; <sup>b</sup>soil from an apple orchard naturally infested with *R. necatrix*; <sup>c</sup>values with a common letter for each location are not significantly different ( $p=0.05$ )

Sede Eliyyahu did not suppress the pathogen. Solarized soils from additional locations in Israel (Rehovot, Bet Dagan and Gamla) had no significant effect ( $p=0.05$ ) in the suppression of the pathogen, as determined by this method, in a separate experiment.

## Discussion

Soil solarization is apparently effective as a pre- and post-planting treatment for controlling *R. necatrix* over an extended period. In Massade, it effectively eradicated the pathogen from soil and root segments, and no reinfestation of soil was apparent after 2 years. In contrast, the pathogen remained viable and caused 60% mortality in young apple trees over a 2-year period in untreated soil. At En Zurim, in an orchard heavily infested with *R. necatrix*, a curative effect in previously diseased trees was observed for 3 years after solarization. Four years after solarization, disease recurred at a low level in solarized trees, whereas all the untreated trees had died. Similar soil temperatures were recorded during solarization in both Massade and En Zurim located in different geographical areas in Israel. Future disease development in trees in solarized soil will indicate how frequently solarization is required to maintain control.

*R. necatrix* is very susceptible to heating and is therefore a good candidate for control by solarization, even in the marginally elevated temperatures that occur in shaded areas and deeper soil layers (Cohen and Szejnberg, 1981). The inoculum of *R. necatrix* was reduced in solarized soil shaded by the canopies of neighbouring trees in the trial described here (Figures 1 and 2). Indirect effects of solarization, such as biological control as shown with *Verticillium dahliae* (Tjamos

and Paplomatas, 1987) and *Rhizoctonia solani* (Elad, Katan and Chet, 1980), accumulation of volatiles and other mechanisms, in addition to direct cumulative heat damage (Katan, 1981; Stapleton and DeVay, 1983; Szejnberg *et al.*, 1987), might contribute to pathogen control. The weakening of fungal propagules by sublethal heating and consequent attack by antagonistic microorganisms (Munnecke, Wilbur and Darley, 1976; Lifshitz *et al.*, 1983; Freeman and Katan, 1988) may also be associated with this phenomenon.

Induced suppressiveness of various soil-borne pathogens in solarized soils from different locations has been observed, suggesting the role of biological factors in control through solarization (Katan, 1985; Greenberger, Yogeve and Katan, 1987; Szejnberg *et al.*, 1987). Two out of seven solarized soils tested here were suppressive to *R. necatrix*. The absence of reinfestation in solarized plots for up to 2 years after solarization, and the continuing control of the disease in a treated orchard over a 3-year period, may be due to induced suppressiveness. Long-term control through soil solarization has been attributed to both inoculum eradication and induced suppressiveness (Katan, Fishler and Grinstein, 1983; Tjamos and Paplomatas, 1988).

Soil solarization is the only disinfection method that can be used in an existing orchard under field conditions (Ashworth and Gaona, 1982; Katan, 1987). It was effective for the control of *R. necatrix* in that: (1) there was no apparent damage to the tree resulting from high soil temperatures; (2) the disease was controlled even in the shaded areas, and (3) the pathogen was eradicated to a soil depth of 60 cm (Szejnberg *et al.*, 1987). Solarization controlled *V. dahliae* in plantings of pistachio (Ashworth and Gaona, 1982), olive (Tjamos, Paplomatas and Biris, 1987) and in almond and avocado (Shabi *et al.*, 1987). It is possible that root rot diseases, such as that caused by *R. necatrix*, may be more amenable to control by solarization than vascular wilts. An increased growth response of woody perennials after post-planting solarization has also been reported (Stapleton and Garza-Lopez, 1988). These results, obtained in various perennial crops and soils and in different climatic regions, suggest that, where the climate is suitable, solarization could be widely applicable to control of soil-borne diseases of perennial crops. A combination of pre- and post-planting treatments may provide even more durable control than obtained here.

## Acknowledgements

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In the framework of our project we studied the effectiveness of solarization on S. rolfsii in two apple orchards: at Givat Ada and at Mazor, both in the central part of Israel. Pathogen populations, disease incidence, and plant growth were followed. Certain aspects were also studied in heated simulation systems in the greenhouse. Most of the studies were carried out in the two existing orchards, but some of the studies were also carried out in a soil to which plants were replanted. Studies at Givat Ada:

The plot was solarized in August 1988, using transparent polyethylene (40  $\mu$ m thick, containing UV absorbants) or IR polyethylene (Ir), which is more efficient in heating the soil. The experiment consisted of four replicates, each with four trees. The trees were 2 years old. During the solarization process, number of irrigations was reduced by 50% in order to avoid excess of soil humidity. The soil was tarped with polyethylene to a distance of 1.8 m of each side of the tree. Disease incidence and pathogen populations were followed in the first year after solarization (1989) and the second year (1990).

Results (Fig. 1) show a trend of a partial reduction by solarization (not statistically significant) in disease incidence in the first year only. In the second year, disease incidence diminished from 25% to 6%. The effectiveness of various soil treatments (solarization, fumigants, Trichoderma), applied prior to planting, on the control of S. rolfsii was examined. No significant effect could be detected. Trichoderma preparation was kindly supplied by Prof. I. Chet.

The effect of soil treatments on viability of sclerotia of the pathogen that are naturally existing in the soil, was followed using the heat sieving technique. Results (Table 1) show a significant reduction in the population of the pathogen, estimated in 1988 and in 1989.



Table 1. Effect of solarization and chemical treatments on population level of Sclerotium rolfsii (Givat Ada). Figures represent no sclerotia / 800 g soil.

Treatment	1988	1989A <sup>(1)</sup>	1989B <sup>(1)</sup>
Untreated	1.25 a	0.5 a	3
Solarization	0 b	0 a	0
M. bromide (MB), 50 g/m <sup>2</sup>	0 b	0 a	0
Solar	0 b	0 a	0
Solar+MB	0 b	0 a	0
Solar (Ir)	0 b	0 a	0
Solar (Ir)+MB	0 b	0 a	0
Vapam	0.25 b	0 a	0

(1) A = Adjacent to the tree trunk; B = At 3 m distance from the tree trunk.

#### Mazor:

In 1988, S. rolfsii disease was detected in a young apple orchard at Mazor. In the subsequent year, solarization was carried out with the following treatments in four replicates:

1. Solarization of young (1-year old) trees, extended solarization (8 weeks starting from June 20, 1989).
2. The same as treatment 1, solarization started only at the end of July (late solarization).
3. Solarization of old (2-year old) trees, extended solarization as above.
4. As treatment 2, using 2 years old trees, as above.

All solarization treatments had respective untreated controls. Disease incidence was recorded during two years: 1989 and 1990.

Results (Fig. 3) show that solarization had no significant effect on disease incidence. At this site, the solarized trees showed severe symptoms of stress. In Givat Ada experiment, the stress symptoms were very mild. Shortly after removal of the polyethylene tarps, the solarized trees recovered.

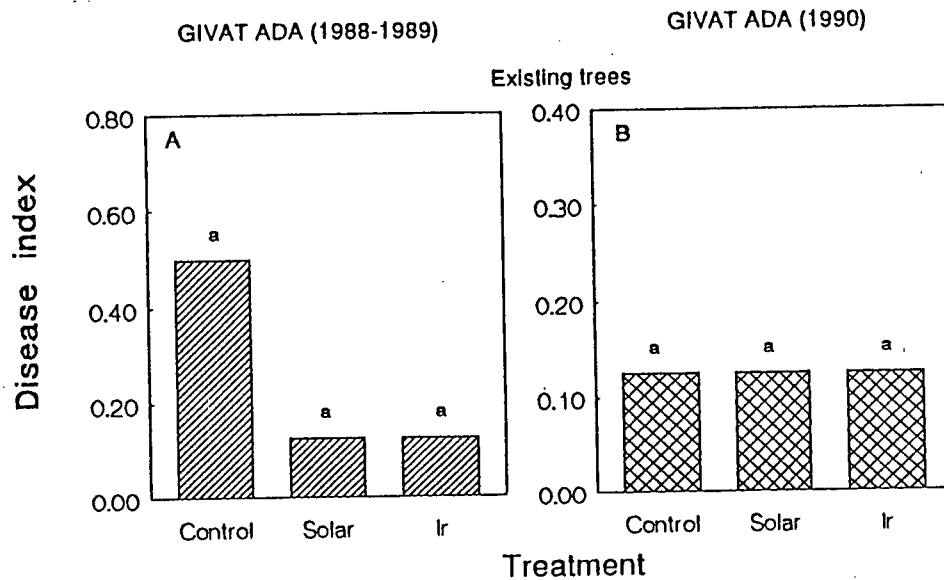


Fig. 1. Effect of soil solarization in 1988 on index of disease caused by *Sclerotium rolfsii* (on a scale of 0-2; 2 = dead tree), determined in 1989 (A) and in 1990 (B). In each year, no significant differences between treatments were obtained. Ir = Polyethylene with higher heating efficiency.

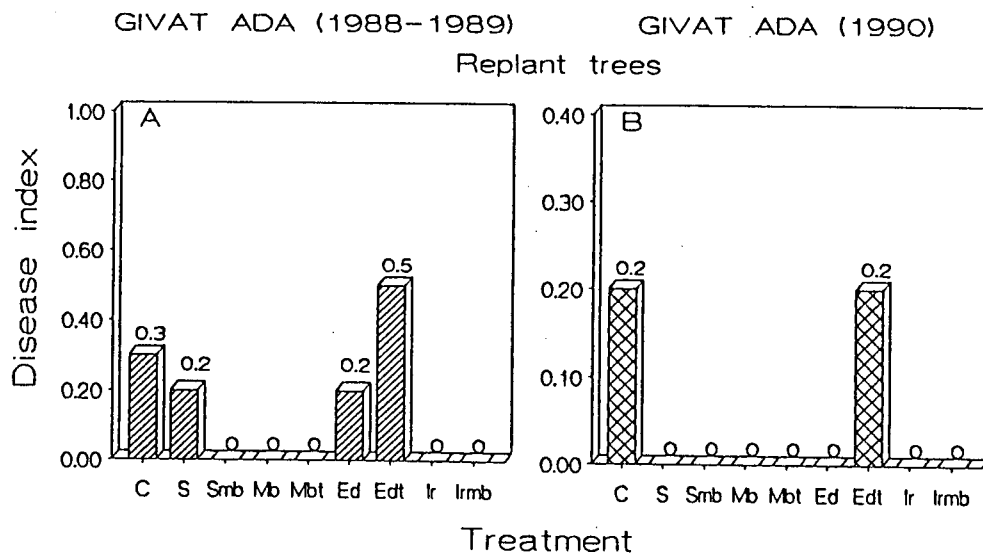


Fig. 2. Effect of solarization in 1988, fumigants and *Trichoderma* on index of disease caused by *S. rolfsii* in replanted apple seedling. Disease evaluations were made in 1988 (A) and in 1989 (B). C = Untreated control; S = Solarization; Mb = methyl bromide at 50 g/m<sup>2</sup>; T = *Trichoderma*; Ed = Vapam; Ir = Solarization with Ir polyethylene.

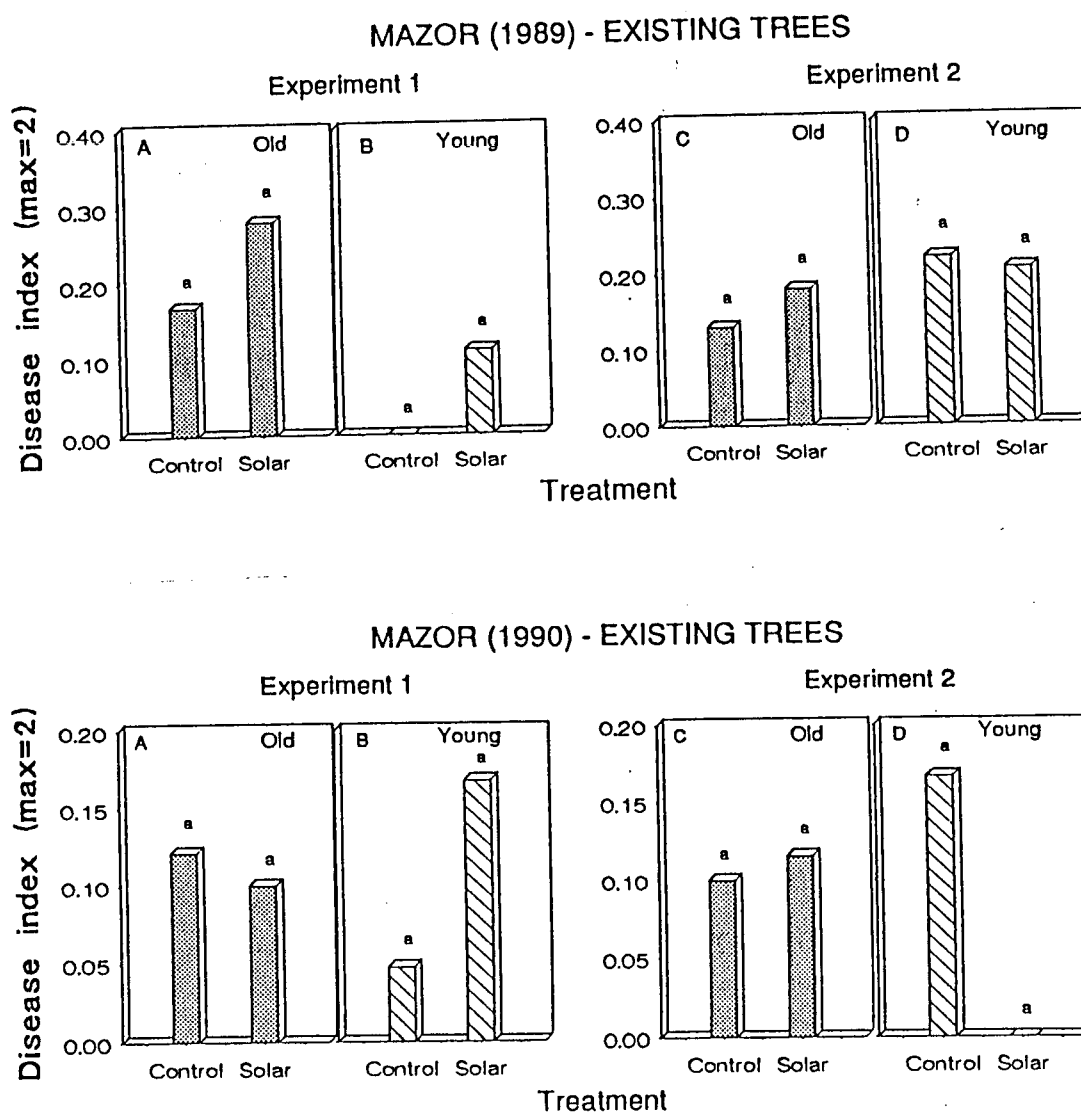


Fig. 3. Effect of soil solarization in existing orchard on disease index (maximum = 2) in an apple orchard infected with *Sclerotium rolfsii*. Solarization was carried out in 1989. Disease incidence was evaluated in 1987 and in 1990. The trees were either young (one year old) or old (two years old). Solarization has no significant effect ( $P = 0.05$ , Duncan's multiple range test).

In an additional experiment, Trichoderma preparation was added to the soil at replanting. Trichoderma populations in the treated plots increased 100 folds.

The effect of solarization on pathogen population was followed. Nylon nets were filled with soil and sclerotia of S. rolfsii and were buried into the soil (solarized and non-solarized plots) to the depths of 10 and 30 cm. These were removed after the termination of solarization and their viability assessed.

Results (Table 2) show that solarization reduced viability of sclerotia by 34-99% depending on soil depths and timing of solarization.

Solarization was more effective at 10 cm depth or if it was extended.

Table 2. Effect of soil solarization on viability of sclerotia of Sclerotium rolfsii. Mazor, 1989.

Treatment	% Germination at depth of	
	10 cm	30 cm
Nonsolar, extended <sup>(1)</sup>	44.9 b <sup>(3)</sup>	42.0 c
Solar, extended <sup>(1)</sup>	0.6 c	1.2 d
Nonsolar, late <sup>(2)</sup>	90.0 a	87.0 a
Solar, late <sup>(2)</sup>	22.1 bc	57.9 b

(1) Solarization was carried out during June-September, 1989.

(2) Solarization was carried out during July-September, 1989.

(3) In each column, denotes no significant difference at  $P = 0.05$ .

Concluding the Sclerotium rolfsii - solarization studies:

Results regarding effectiveness of solarization were variable. Pathogen population was effectively reduced by solarization (Tables 1 and 2).

However, this was not reflected in a comparable success in disease control, in spite of the fact that usually, the population of this

pathogen is concentrated in the upper layer of the soil. The standard approach in Israel is to mulch apple orchards with straw. The possibility that straw may interact with pathogen was further studied.

### 3. Solarization for the control of other soilborne diseases in trees

#### (a) Almond - Verticillium wilt:

An almond orchard which was infected with V. dahliae was chosen for solarization studies, before the initiation of this project. Results (Fig 3A) show that solarization was effective in reducing disease incidence during three years. In the following year, disease incidence was naturally diminished to zero in both solarized and non-solarized plots. The phenomenon has been reported in the past. The importance of solarization in this case is in protecting the trees during the early stages of disease development.

The above orchard was planted either after potatoes (which were heavily infected with the pathogen) or after wheat (free of pathogen). Results (Fig. 3B) show that potato history increased disease incidence.

#### (b) Avocado - Phytophthora cinnamomi:

An avocado orchard was infected with the soilborne pathogen P. cinnamomi. Earlier experiments had shown that solarization is very effective in controlling the pathogen even to the depth of 80 cm. We therefore studied the possibility that solarization can be effective in controlling this pathogen in an existing orchard. In summer 1988, four rows of avocado, each 125 X 5 m, were pruned, the branches cut and the soil solarized. In the following months, pathogen population in the soil was assayed. It was found that solarization reduced pathogen population to undetectable levels. However, the effect on disease control could not be measured since the trees were killed by frost the following winter. This led to the conclusion that in future studies such trees should not be severely pruned before solarization.

# VERTICILLIUM WILT IN ALMOND EFFECT OF SOIL SOLARIZATION

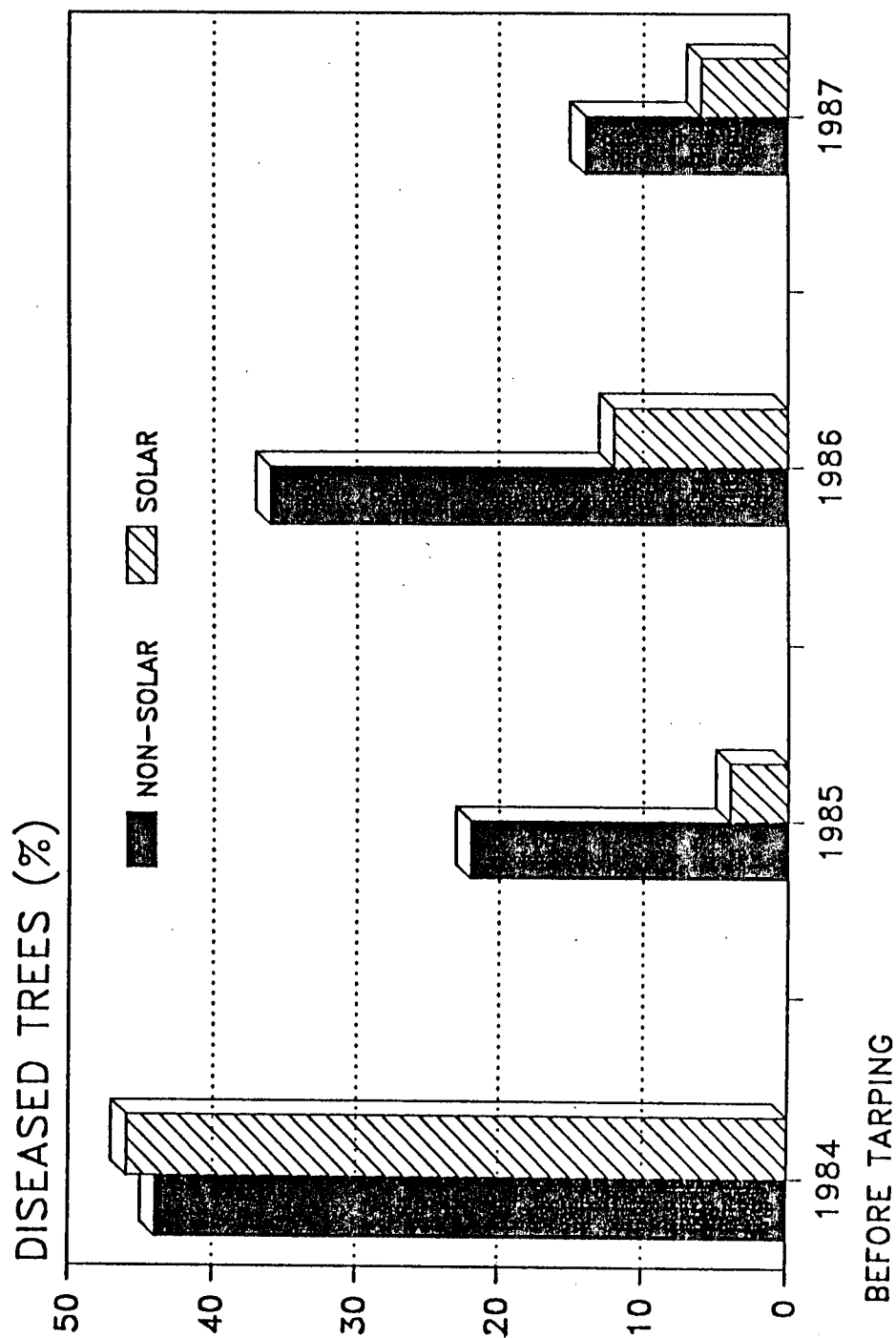


Fig. 3A. Disease incidence (Verticillium wilt) in almond orchard, as affected by solarization (Lahav experiment).

# VERTICILLIUM WILT IN ALMONDS

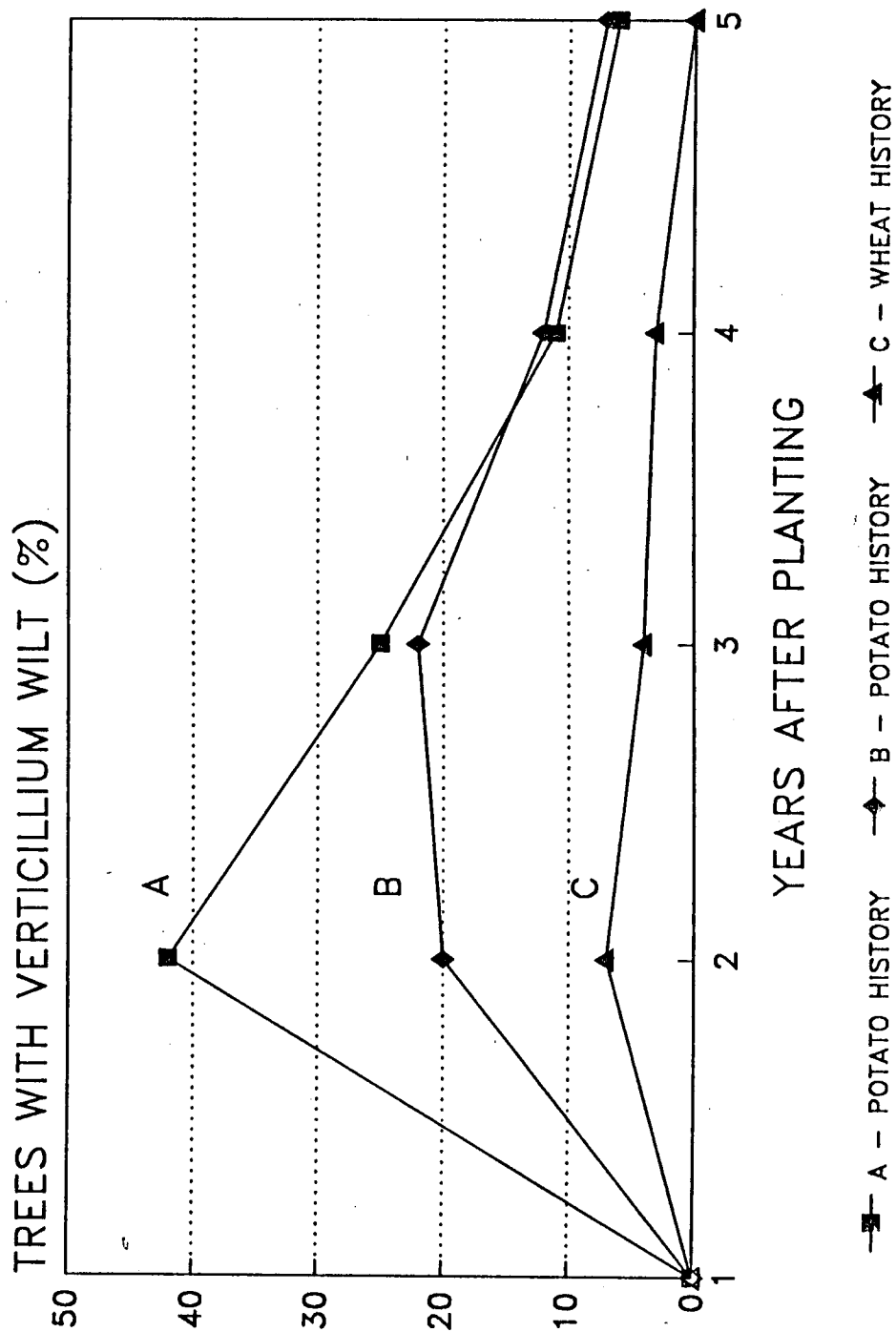


Fig. 3B. Disease incidence (Verticillium wilt) in almond orchard, as affected by crop history (Lahav experiment).

(c) Apples - at Golan heights.

A new severe disease attacked apple orchards in the Golan heights, especially in the vicinity of Keshet. The trees showed symptoms of loss of turgor, and leaf desiccation, finally leading to the death of the trees. The causal organism of this disease is not yet determined but it is possible that the disease is caused by a soilborne pathogen.

In summer 1988, we solarized in four replicates an orchard which was severely affected by this disease. In the following two years, growth of the trees was recorded. Solarization improved growth of the foliage and the roots and reduced disease severity. This finding supports the (yet unproven) hypothesis that this disease is caused by a soilborne pathogen.

(d) Replant disease in peaches and apples (in cooperation with A. Gur, Y. Luceti and Y. Cohen):

Replanting trees in a plot in which an old orchard has been grown previously, usually results in a poor growth of the replanted trees. This phenomenon is referred to as the "Replant disease". Preplant soil fumigation, e.g. with methyl bromide or chloropicrin, frequently controls this disease. We therefore carried out experiments to study the effectiveness of preplant solarization (as compared to fumigation) in controlling the replant disease in peach and apples.

One experiment with peaches was carried out in Nir Banim. Results from the 1988 season are given in Table 3. They show that fumigation with methyl bromide and chloropicrin had a significant effect, compared to the control. Solarization was less effective than methyl bromide + chloropicrin.



Table 3. Effect of preplant fumigation or solarization on growth and yield of peaches replanted in a peach orchard. Nir Banim, 1988.

Treatment <sup>(1)</sup> and rate (g/m <sup>2</sup> )	Trunk circumference (cm)	Pruned weight (Kg)	Yield Kg/plot
Untreated	30.0 b <sup>(2)</sup>	5.39 b	37.2 b
Solarization	31.6 ab	5.97 ab	39.6 ab
Solar+MB 25	31.6 ab	6.27 ab	41.0 ab
MB 50	31.0 ab	6.25 ab	41.0 b
MB 50+Cp 38	33.4 a	7.41 b	44.5 a

(1) MB - methyl bromide; CP = chloropicrin

(2) In each column, numbers with a common letter do not differ significantly ( $P = 0.05$ ).

Replanting experiments were also carried out with apples at Yiron, in a plot in which apples have been grown in the past. Solarization or fumigation, were carried out in summer 1988, as specified. Results (Table 4) show that this plot had no severe replant disease. In only one case, the combined fumigant treatment significantly increased the circumference (in 1990).

Table 4. Effect of soil disinfestation as a preplant fumigation or solarization on growth and yield of replanted in an apple orchard. Yiron, 1988-90.

Treatment <sup>(1)</sup> and rate (g/m <sup>2</sup> )	Trunk circumference (cm)			Yield (Kg/m <sup>2</sup> )
	1988	1989	1990	
Untreated	83.2 a <sup>(2)</sup>	142 a	194 b	2.24 a
Solarized	87.4 a	141 a	190 b	2.12 a
Formalin	83.8 a	134 a	189 b	1.81 a
CP 38+MB 50	90.6 a	151 a	208 a	2.22 a
MB 50	87.8 a	145 a	199 ab	2.37 a

(1) CP = chloropicrin; MB = methyl bromide

(2) In each column, figures with a common letter are not significantly different ( $P = 0.05$ ).

4. Soil temperatures in the solarized orchards

Soil temperatures in solarized orchards are much affected by tree size, shape and position. Temperatures in solarized orchards, at various soil depths and positions, and solar radiations were recorded in 1989 and 1990 at two sites: Mazor and at the experimental farm of the Faculty of Agriculture at Rehovot ("HAVA"). Results (Figs 4-7) show that, as expected, temperatures were lower at lower soil layers and that the maximal temperatures were attained later in the day. Soil temperatures were dependent on tree position.

5. Effect of solarization on plant growth and physiology

Solarization was carried out in 1989 in an orchard with two years old apple plants. Plant growth and physiological parameters were followed during and after solarization. Two cultivars were used.

Plant growth was adversely affected by solarization. Chlorosis of solarized plants was evident. This was expressed in chlorosis intensity (Fig. 8). The cultivar Starking was more heat-sensitive than CV Anna. Photosynthesis, and transpiration were also affected by solarization (Fig. 9), and again, Starking was more adversely affected.

The adverse effects of heating on apple plants were also demonstrated in a heating system at 40 C and 45 C. This system simulates natural soil solarization (Figs. 10 and 11).

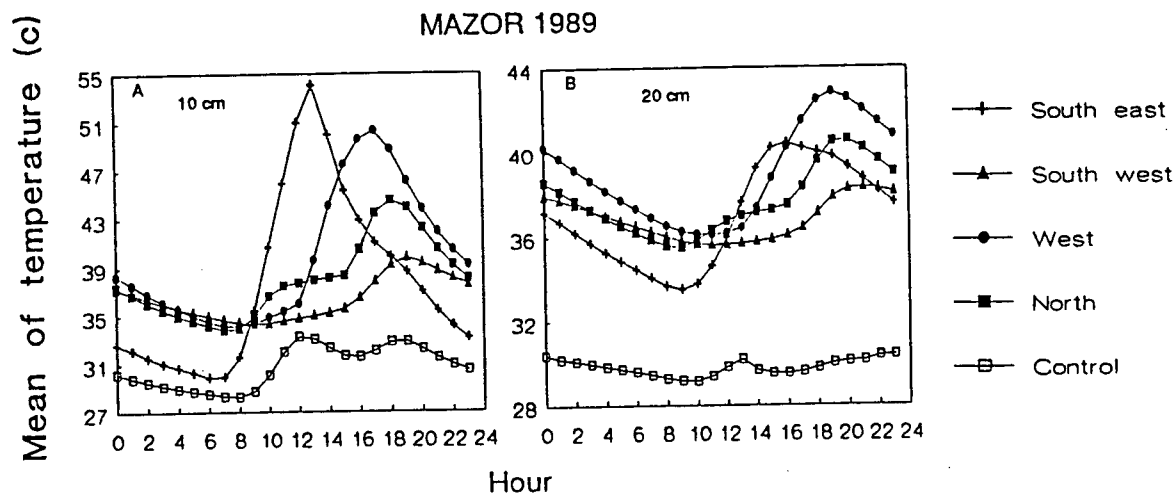


Fig. 4. Effect of soil solarization in an apple orchard on temperatures at 10 and 20 cm depths. Measurements were taken at various positions at 70 cm distance from the tree. Mazor, 1989.

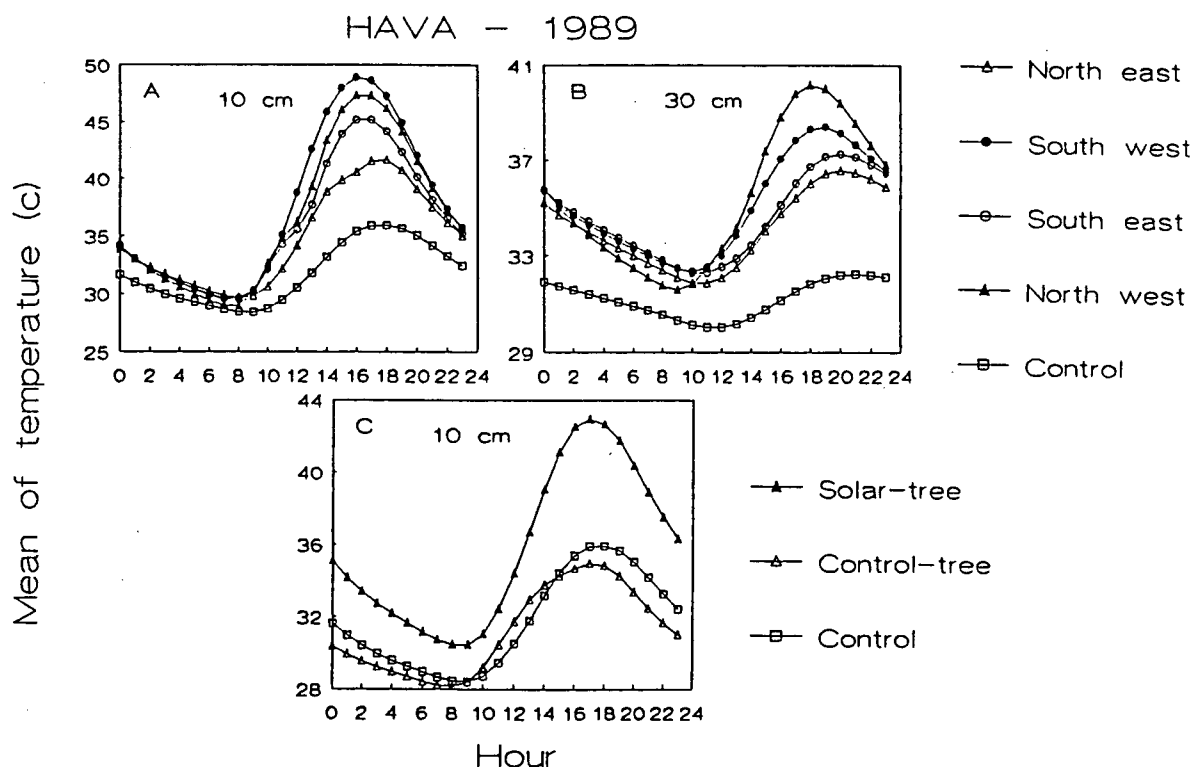


Fig. 5. Effect of soil solarization in an apple orchard on temperatures at 10 and 20 cm depths. Measurements were taken at various positions at 50 cm distance from the tree. The experimental farm at Rehovot, 1989.

## HAVA - 1990

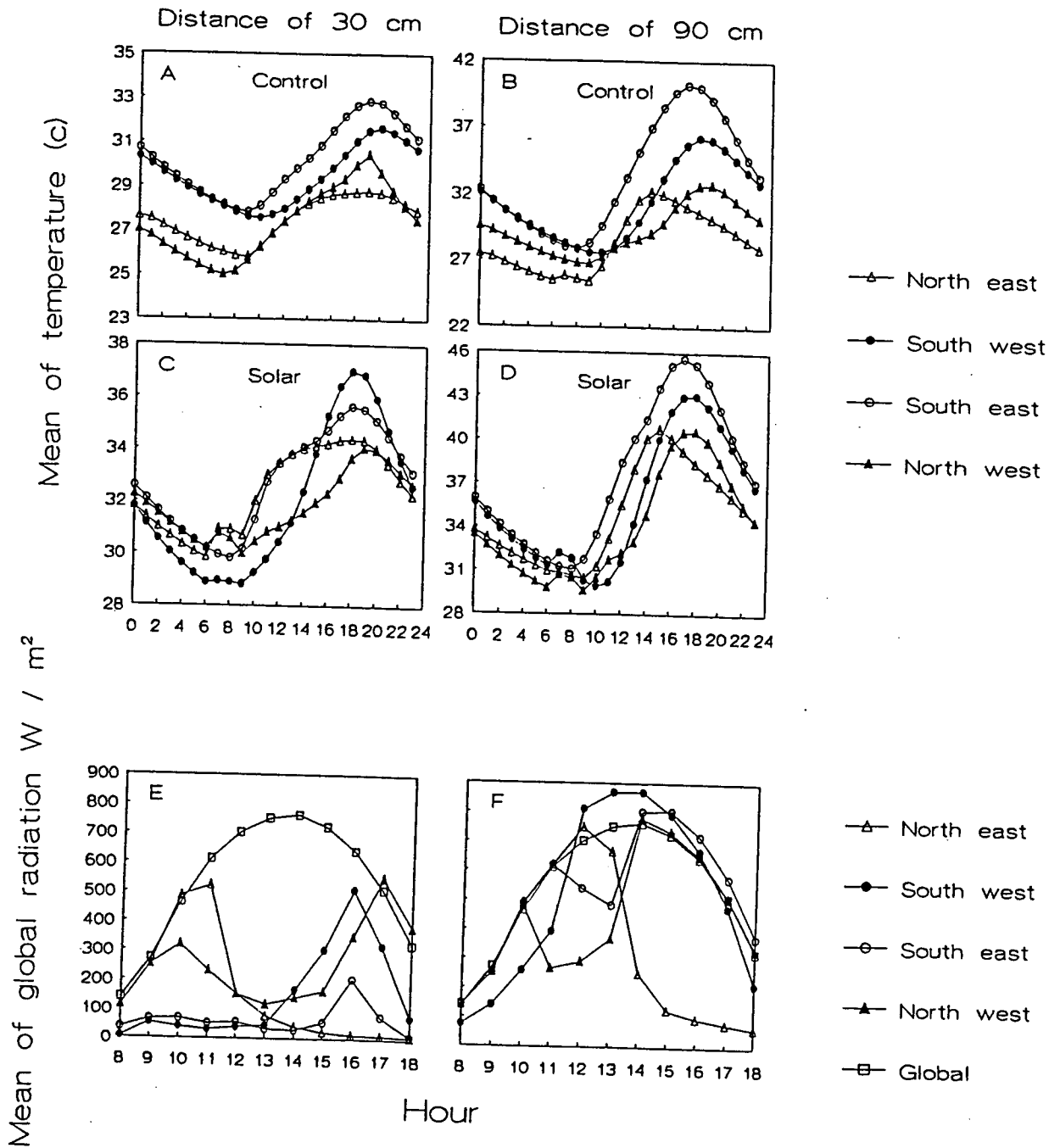


Fig. 6. Soil temperatures (at 10 cm depth) and global radiation in a solarized orchard, at various positions. Rehovot 1990.

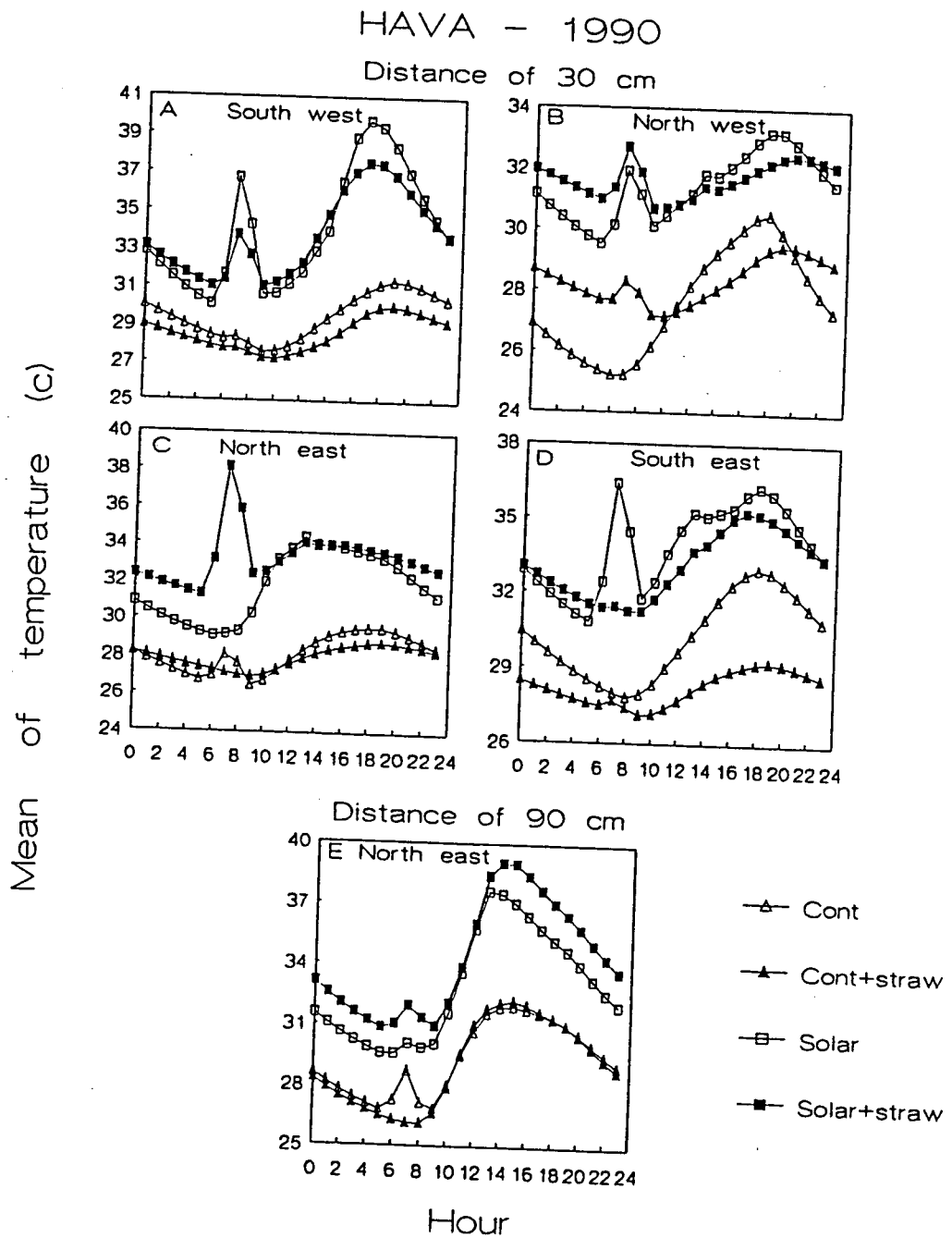


Fig. 7. Effect of soil solarization in an apple orchard on temperatures at 10 and 20 cm depths, at various positions at 30 and 90 cm distance from the tree. Rehovot, 1990.

After the removal of the plastic tarp, recovery of the plants in the solarized plots was evident. In the following spring (1990), growth of the plants in the solarized plots was improved (Fig. 12). The differences in growth were still evident one year later (Table 5). This phenomenon is similar to that described as increased growth response in annual crops (see below).

Table 5. Long term effect of postplant solarization on growth of apple plants, two years after solarization. <sup>(1)</sup>

Treatment	Plant height (cm)
Unsolarized	154.6 b
Solarized	171.9 a

(1) Solarization was carried out on July 1989. Measurement were taken in June 1991. Differences between treatments are significant ( $P = 0.05$ ).

6. Volatiles in the relation to the effect of solarization on *Phytophthora cinnamomi* buried in closed and open containers in soil

In a previous experiment soil solarization was found to be very effective mean in controlling *Phytophthora cinnamomi*, the devastating soilborne pathogen. The experiment was performed in avocado grove heavily infected with root rot disease. Solarization was carried out for four and a half weeks, during August and September. At the end of the experiment soil samples were taken from various depths, from both solarized and non-solarized plots. In the treated plots, all samples, including those taken from depths of 70 cm were pathogen free, while with those taken from non-solarized plots the level of the pathogen did not change significantly. In many experiment it could be demonstrated that at depth of 70 cm, the temperature curves during day and night are nearly identical with both solarized and non-solarized plots. The maximal temperature, down at depth of 70 cm is only one degree centigrade higher in the solarized plot. Based on those figures it was suggested that at least at deeper layers other factors, beside heat, contribute to the excellent control results obtained. It was further assumed that among the various factors that are altered during

solarization, production of volatile toxic materials that are trapped under the plastic layer, might contribute to the effectiveness of the control in deeper soil layers. To test this hypothesis, an experiment was designed in which contaminated soil samples enclosed in weaved plastic sleeves were buried 35 and 70 cm, in two neighboring plots, 5 replicate at each depth. However, at each location two samples were buried together. One enclosed in perforated, rigid, plastic container, to enable exposure to soil atmosphere and the other in sealed container. Solarization continued for 6 weeks. During that period the non-solarize plot was irrigated regularly. The contaminated soil was prepared by blending millet seeds colonized with Phytophthora cinnamomi (isolated from avocado feeder roots) in water. The suspension was mixed thoroughly with non-infested soil. The concentration of the pathogen was determined before and after infested the experiment by mixing 10 g of soil sample with 10 ml of water-agar (0.2%) for ten minutes. The samples were further diluted 1:10. From the first and second diluted suspensions, five replicates, 1 ml each, were dispersed on PARPH selective medium, incubated for 48 h in 23 C before rinsed with water. The number of P. cinnamomi colonies were counted after additional 24 and 48 h. At the end of the experiment the presence or absence of the pathogen was ascertained using P. indica seedlings as trap plants (5 g of soil samples in 500 ml of water). The presence of one to 10 zoospores in the soil normally causes typical discoloration symptoms.

The effect of toxic volatile material in helping controlling pathogen could be demonstrated if the number of viable propagules detected was higher in the opened containers as opposed to closed ones. This could not be demonstrated (Table 6) therefore the working hypothesis could not be ratified.

In the current experiment the effectiveness of solarization in controlling P. cinnamomi was again demonstrated, though with one replicate (Table 6), at the deeper location, complete eradication was not achieved. Trapping the pathogen with P. indica seedlings (not presented), was found to be in this case less sensitive method compared to the direct isolation technique. In no instance the presence of the pathogen could not be demonstrated with P. indica seedlings when the

result obtained by the direct isolation technique revealed the presence of the pathogen but those findings could not be backed up with P. indic seedlings.

Table 6. Effect of soil solarization, and volatile escape on viability of Phytophthora cinnamomi

<u>No. propagules / 0.5 g soil, at depth</u>				
<u>Treatment</u>	<u>35 cm</u>		<u>70 cm</u>	
	<u>Closed container</u>	<u>Open cont.</u>	<u>Closed container</u>	<u>Open cont.</u>
Non-solarized	*19	19	10	12
	13	2	13	9
	8	20	19	11
	12	10	16	4
	15	16	4	5
Average	12	13.4	12.4	8.5
Solarized	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	6
Average	0	0	0	1.2

\* Each figure represent an average of 3 replicates.



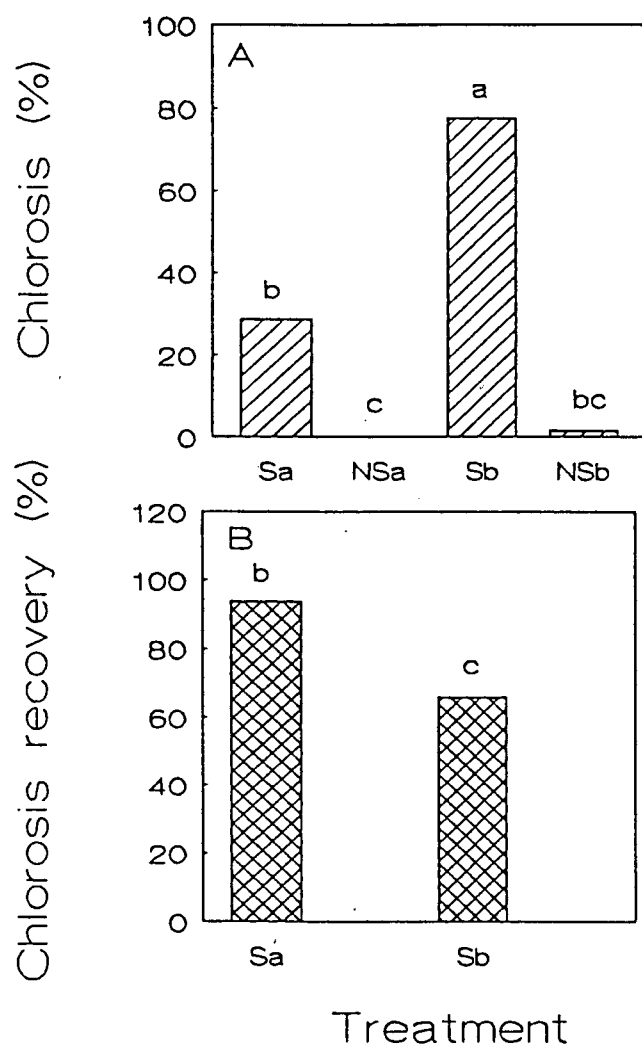


Fig. 8. Effect of soil solarization on incidence of chlorosis in apple plants during solarization (A) and on their recovery 40 days after the removal of the plastic tarp. Two cultivars were used: Anna (a) and Starking (b). S = Solarized; NS = Nonsolarized.

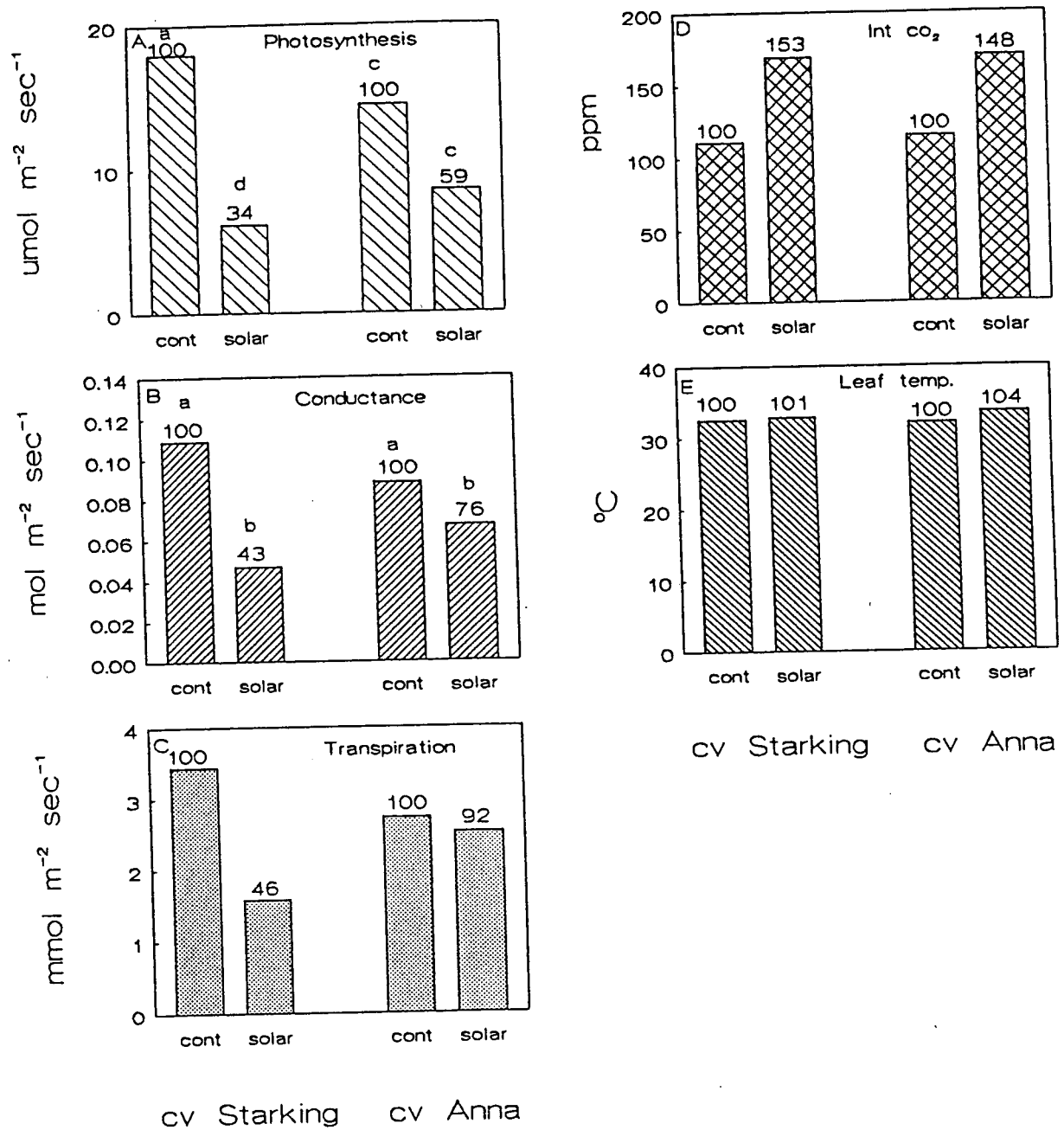
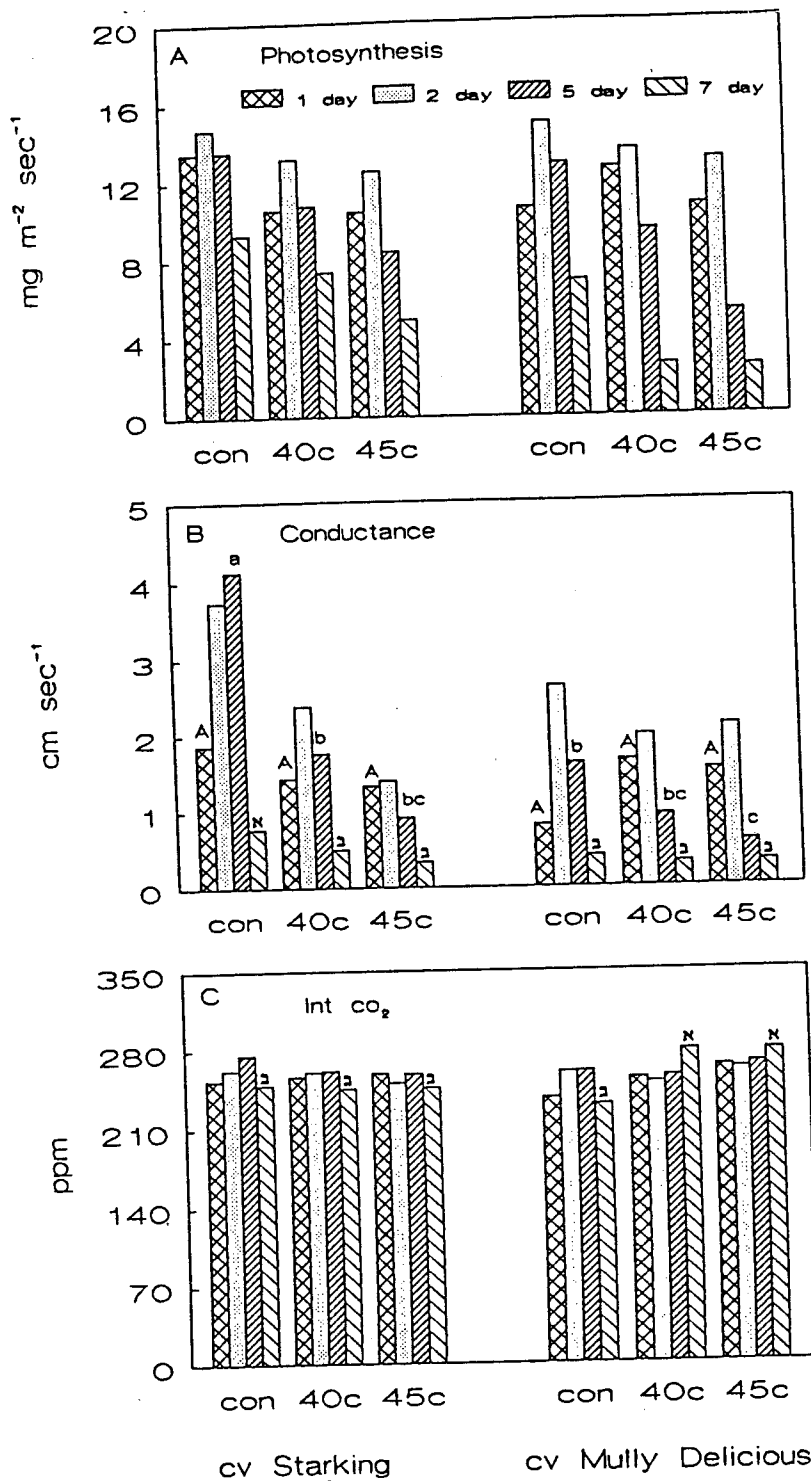


Fig. 9. Effect of soil solarization of apple plants on physiological parameters: photosynthesis (A), stomatal conductiveness (B), transpiration (C), internal  $\text{CO}_2$  (D) and leaf temperature (E). cnt = unheated control.



ניתוח שונות

פרמטר	זמן	אינטראקציה	השפעה עיקרית
ימים	חיסוס	חיסוס	זן
זן	45	40	28
ב	ב	ב	ב
פוטוסינתזה	a a	a a a	-
	a a	a a a	-
	a a	c b a	-
	b a	b b a	-
פוליכות			+
	a a	b ab a	-
			+
			+
CO <sub>2</sub> פנימי	a a	a a a	-
	a a	a a a	-
	a a	a a a	-
			+

Fig. 10. Effect of heating in a simulation system (at 40 C and 45 C) on physiological parameters of apple plants. Measurements were taken after 1,2,6 and 7 days of heating.

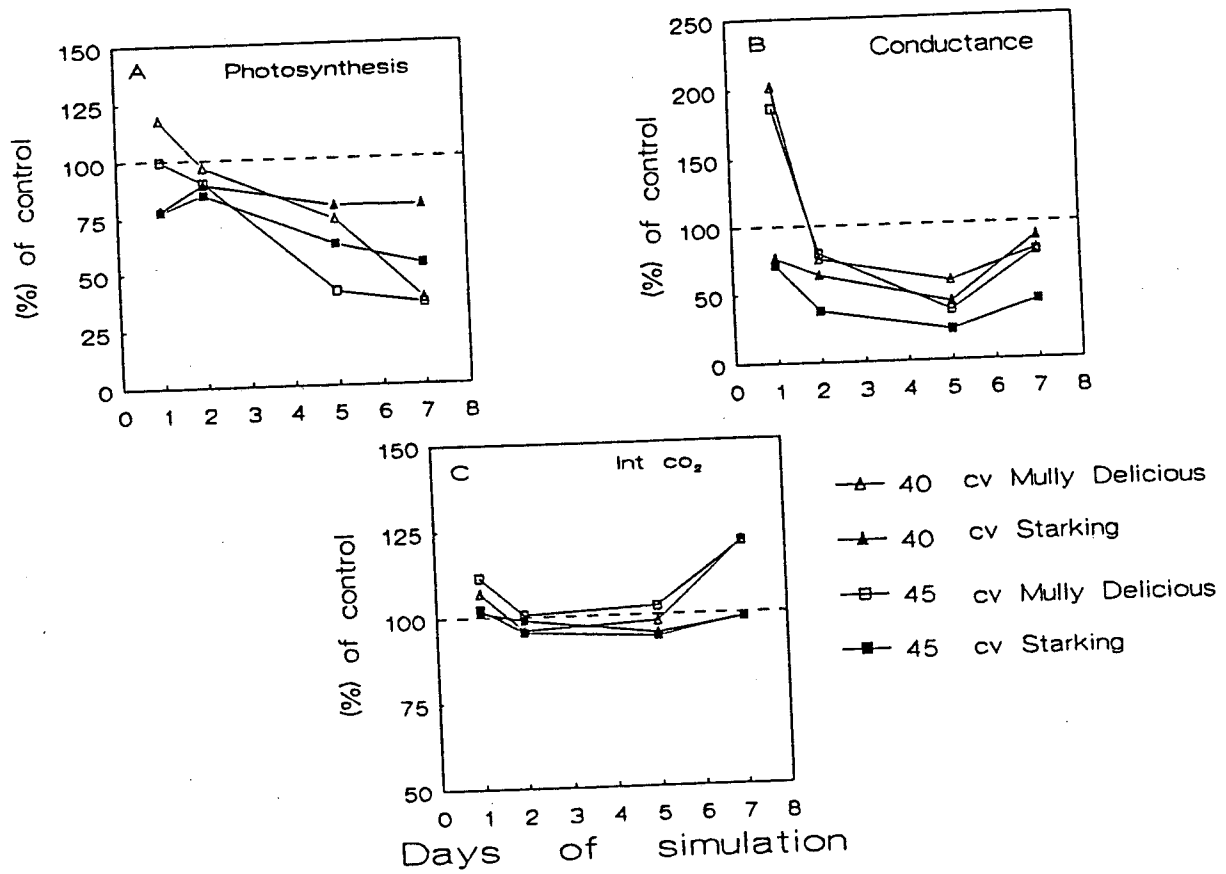


Fig. 11. Effect of heating (as specified in Fig 10) on apple growth, expressed as % of nontreated control.

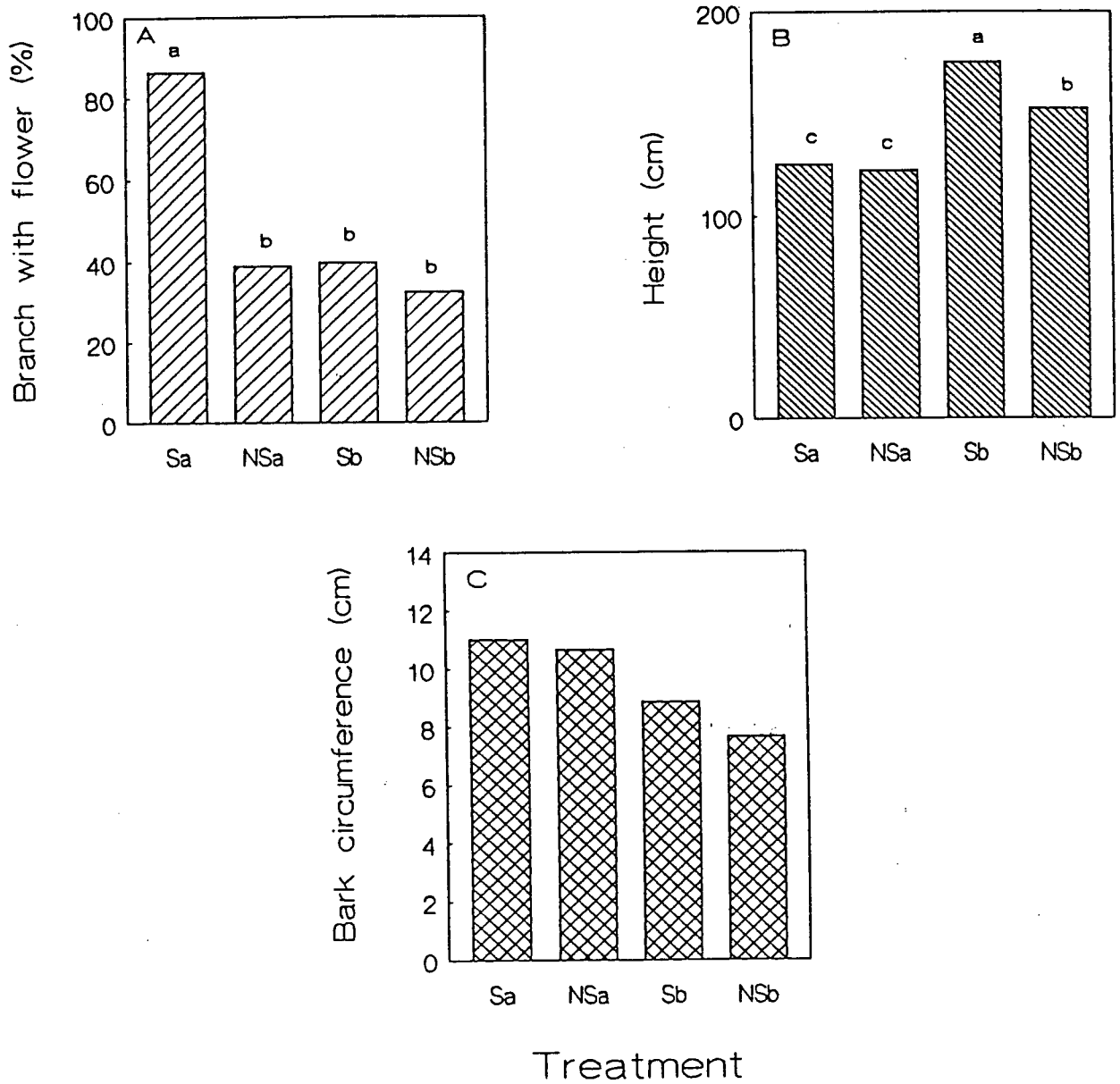


Fig. 12. Growth of apple plants, as affected by solarization, one year after the termination of solarization in 1989. S = Solarization; NS = Nonsolarized; a = CV Anna; b = CV Starking.

## 7. Increased growth response

Numerous studies carried out since the beginning of this century had shown that, frequently, growth of plants growing in disinfested soils (fumigated or steamed) was improved, even in the absence of known pathogens. This phenomenon is one of the positive side effects of soil disinfestation and is attributed to chemical, physical, or biological changes that occur in soil during and after disinfestation. For example increases in soluble minerals nutrients were found in fumigated and solarized soils.

Plant growth may be positively or adversely affected by soil organisms. Certain strains of fluorescent pseudomonades are beneficial organisms, whereas some harmful strains of fungi, e.g., Penicillium spp., and bacteria are referred to as minor pathogens. The latter invade roots, meristemic tissues, rootlets, and root hairs, reducing root activity and plant vigor. Soil disinfestation may alter populations or activity of soil organisms and, consequently, improve or suppress plant growth. Soil solarization affects microbial activities in soil and may result in increased antagonistic activity and induced soil suppressiveness. Solarization increases populations of fluorescent pseudomonades in the rhizosphere of tomato plants grown in container media. In the present study, we investigated the effect of soil disinfestation, mainly solarization, on the growth response in several crops with a variety of soils; the effect of solarization on microbial populations in nonrhizosphere and rhizosphere soil and in plant roots under greenhouse and field conditions; and the possible relationship of microbial change to increased growth response. It appears that increased growth response was detected in solarized trees (Fig 12, Table 5 and the US section of the report). We therefore studied this possible improvement of microbial phenomena on growth improvement. We used annual crops as model plants

### Summary of the results

Enhancement of plant growth in the absence of known pathogens was studied on solarized soils and, to a limited extent, in methyl bromide- and in metham-sodium-treated soils. Increased growth response, expressed in increased dry weight of tomato plants in treated over untreated soil

in the greenhouse, was evident in most of the 24 tested soils from various location in Israel. Increased growth was also evident in field experiments and in an artificially heated soil. Regression analysis showed a significant, inverse relationship between soil pH and increase growth and between soil pH and population densities of fluorescent pseudomonades in the rhizosphere. Solarization reduced bacterial and fungal population densities to a depth of 90 cm, whereas actinomycetes were less affected.

Thermotolerant bacteria and fungi also were reduced by solarization. Population densities of fluorescent pseudomonades were increased up to 130-fold in the rhizosphere of plants in solarized soils, although these bacteria are heat sensitive. Solarization drastically reduced population densities of total fungi in the rhizosphere and roots, especially Penicillium pinophilum, which causes plant stunting in greenhouse tests, and Pseudomonas putida, P. fluorescent, and P. alcaligenes, which were recovered from solarized soil, stimulated growth of plants. Solarization increased the frequency of recovery of bacteria showing antagonistic activity from the rhizosphere and roots of tomato plants. Further details are given in the enclosed article.

8. Combining heating and inorganic and organic amendments for improved control of *S. rolfii*

Previous studies showed that combining heating with certain amendments, especially those which have adverse effects on the pathogen has a synergistic effect. We therefore, studied the effect of nitrogenous compounds on cabbage residues since these have been reported to affect *S. rolfii*. With some combinations, the control was very effective. For example, combining heating at 45 C with ammonia at 500 ppm resulted in complete control of the pathogen (Fig. 13). Combining cabbage residues (at 1000 ppm) with heating at 45 C, completely eradicated the pathogen (Fig 14). These results denote that some suitable combinations may be very valuable for improving solarization under marginal conditions.

## Involvement of Fluorescent Pseudomonads and Other Microorganisms in Increased Growth Response of Plants in Solarized Soils

A. Gamliel and J. Katan

Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel.

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### ABSTRACT

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Enhancement of plant growth in the absence of known pathogens was studied in solarized soils and, to a limited extent, in methyl bromide- and in metham-sodium-treated soils. Increased growth response, expressed by increased dry weight of tomato plants in treated over untreated soils in the greenhouse, was evident in most of the 24 tested soils from various locations in Israel. Increased growth also was evident in field experiments and in an artificially heated soil. Regression analyses showed a significant, inverse relationship between soil pH and increased growth and between soil pH and population densities of fluorescent pseudomonads in the rhizosphere. Solarization reduced bacterial and fungal population densities to a depth of 90 cm, whereas actinomycetes were less affected.

Thermotolerant bacteria and fungi also were reduced by solarization. Population densities of fluorescent pseudomonads were increased up to 130-fold in the rhizosphere of plants in solarized soils, although these bacteria are heat sensitive. Solarization drastically reduced population densities of total fungi in the rhizosphere and roots, especially *Penicillium pinophilum*, which causes plant stunting in greenhouse tests, and *Pythium* spp. Isolates of fluorescent pseudomonads, identified as *Pseudomonas putida*, *P. fluorescens*, and *P. alcaligenes*, which were recovered from solarized soil, stimulated growth of tomato plants. Solarization increased the frequency of recovery of bacteria showing antagonistic activity from the rhizosphere and roots of tomato plants.

**Additional keywords:** biological control, induced suppressiveness, minor pathogens, plant growth-promoting rhizobacteria.

Soil disinfestation is a preplanting treatment used for controlling harmful soilborne organisms. Disinfestation of pathogen-infested soils, by steaming, fumigation, or solarization usually results in the reduction of populations of these organisms and in improved plant growth and yield. However, increased growth response of plants in disinfested soils frequently was observed even in the absence of known pathogens (2,5,15,19,20,31-34). This phenomenon is one of the positive side effects of soil disinfestation and is attributed to chemical, physical, or biological changes that occur in soil during and after disinfestation. For example, increases in soluble mineral nutrients were found in fumigated (2,20,34) and solarized (5,33) soils.

Plant growth may be positively or adversely affected by soil organisms. Certain strains of fluorescent pseudomonads are beneficial organisms (17,18,27), whereas some harmful strains of fungi, e.g., *Penicillium* spp., and bacteria are referred to as minor pathogens (23,26,35). The latter invade roots, meristematic tissues, rootlets, and root hairs, reducing root activity and plant vigor. Soil disinfestation may alter populations or activity of soil organisms and, consequently, improve or suppress plant growth.

Soil solarization affects microbial activities in soil and may result in increased antagonistic activity and induced soil suppressiveness (9,32). Solarization increases populations of fluorescent pseudomonads in the rhizosphere of tomato plants grown in container media (8). In the present study, we investigated the effect of soil disinfestation, mainly solarization, on the growth response in several crops with a variety of soils; the effect of solarization on microbial populations in nonrhizosphere and rhizosphere soils and in plant roots under greenhouse and field conditions; and the possible relationship of microbial changes to increased growth response.

### MATERIALS AND METHODS

**Soils and disinfestation.** Soils of various textures were collected from fields at 24 locations in Israel during 1984-1988. The pH of these soils ranged from 6.9 to 8.57, with 3-58% clay, 0-45% silt, 9.2-96% sand, and 0.1-3.4% organic matter. One soil (Machanayim) was exceptional in that it contained 25% organic matter. Soils were untreated or disinfested by solarization, fumigation with methyl bromide at 55 g/m<sup>2</sup>, or treatment with metham-sodium at 600 L/ha. Soil samples were collected from the upper 20-cm layer (after removing the top 2-3 cm) from either experimental plots (8 × 15-20 m) or commercially disinfested fields, in which the whole field was disinfested; five plots were left untreated. In experimental plots, soil samples were taken from the four replicates of each treatment. In commercial fields, soil samples were taken randomly from five sites in the disinfested field and from the five plots that were left untreated. In some experiments, soil samples were also taken to a depth of 90 cm with a core auger (5-cm i.d.). Disinfestation was accomplished under field conditions according to standard procedures. Solarization was carried out either manually or mechanically by mulching preirrigated soil with transparent polyethylene sheets (30-50 µm thick) in July-August for 35-55 days. Typical temperatures of the solarized soils at depths of 10 and 30 cm were 44-48 and 36-40 °C, respectively. The temperatures of the corresponding unsolarized soils were 7-12 °C lower. Fumigation with methyl bromide was carried out by the hot-gas technique with commercial equipment. Metham-sodium was applied using a sprinkler irrigation according to the standard procedure.

**Simulation of soil solarization.** Artificial heating of soil was done in specially designed and modified Wisconsin soil-temperature tanks, as reported previously (9,36). The heating system in the simulation tank resulted in the gradual warming of the soil to a maximum temperature of 45 °C for a period of



approximately 4 hr every day, after which the temperature dropped gradually to 30–34 °C. The daily heating course of the soil was similar to that in the upper 10-cm layer of soil during solarization in Israel. Two-liter cylindrical glass jars (25 cm high, 12 cm diameter) were filled with soil moistened to field capacity. Jars were sealed with polyethylene sheets to prevent evaporation and maintained for 42 days in the tanks. Untreated soil was prepared similarly and kept in a shaded part of the greenhouse at temperatures of 22–28 °C.

**Plants.** Tomato (*Lycopersicon esculentum* Mill. 'Rehovot 13'), cotton (*Gossypium barbadense* L. 'Pima S-5' and 'Pima F-27'), pepper (*Capsicum annuum* L. 'Maor'), eggplant (*Solanum melongena* L. 'Black Beauty'), corn (*Zea mays* L. 'Jubilee'), and sorghum (*Sorghum bicolor* L. '610') were used in these experiments.

**Media.** Selective media were used for enumeration of microbial populations in nonrhizosphere and rhizosphere soils and root

tissues. Nutrient agar (N) (7) was used for total bacteria; King's B agar medium (KB) (7), modified by the addition of 100 mg/L of cycloheximide, 50 mg/L of ampicillin, and 12.5 mg/L of chloramphenicol (30), plus 5 mg/L of pentachloronitrobenzene (PCNB) to suppress *Rhizopus*, was used for fluorescent pseudomonads; Martin's agar (7) was used for total fungi; Martin's agar, supplemented with 5 mg/L of PCNB was used for enumeration of *Penicillium pinophilum*; colloidal chitin medium (11) was used for enumeration and isolation of actinomycetes; sucrose-asparagine agar (28) was used for isolating *Pythium* spp. from roots; peptone-PCNB medium (7), acidified with 1 ml/L of 90% lactic acid and supplemented with 250 mg/L of chloramphenicol instead of streptomycin, was used for *Fusarium* spp.; potato-dextrose agar (PDA) (7) was used for culturing fungi and inoculation tests.

**Assays of plant growth. Greenhouse experiments.** Plants were grown in 10-cm-diameter pots filled with the test soils. With the

TABLE 1. Effect of soil solarization, heating in a simulation system for 42 days, treatment with metham-sodium, or fumigation with methyl bromide on growth of plants<sup>a</sup>

Location	Plant tested	Soil treatment	IGR (%) <sup>b</sup>		Number of experiments	
			Average	Range	Total <sup>c</sup>	Significant <sup>d</sup>
Barqay	Tomato	Solarization	50	3–90	3	2
	Sorghum	Solarization	41	38–45	2	2
Besor	Tomato	Solarization	24	...	1	1
Bet Dagan	Tomato	Solarization	57	23–96	5	4
	Tomato	Simulation	33	...	1	1
	Sorghum	Solarization	22	15–30	2	1
Bet Hananya	Tomato	Solarization	23	55–90	2	2
Bet HaShitta	Tomato	Solarization	68	59–77	2	2
Gamla	Tomato	Solarization	52	15–89	2	1
	Tomato	Methyl bromide	20	12–28	2	1
Eden	Tomato	Solarization	52	...	1	1
	Cotton	Solarization	74	25–128	3	3
	Cotton	Simulation	43	30–56	2	2
En Zurim	Sorghum	Solarization	131	...	1	1
Gaza	Tomato	Solarization	64	50–78	2	2
	Tomato	Methyl bromide	20	...	1	0
Gilgal	Tomato	Solarization	45	34–57	2	2
	Tomato	Methyl bromide	51	25–77	2	1
Kefar Warburg	Tomato	Solarization	30	...	1	1
Kefar Yedideya	Tomato	Solarization	29	...	1	1
	Sorghum	Solarization	134	...	1	1
Lakhish	Tomato	Solarization	9	...	1	1
Maale Gilboa	Tomato	Solarization	15	2–28	2	1
Machanayim	Tomato	Solarization	110	97–123	2	2
Magen	Tomato	Solarization	67	53–81	2	2
Qidron	Tomato	Solarization	34	...	1	1
	Tomato	Methyl bromide	42	...	1	1
Rehovot	Tomato	Solarization	53	23–191	5	5
	Tomato	Simulation	40	27–54	2	2
	Sorghum	Solarization	32	25–39	2	2
	Pepper	Solarization	108	...	1	1
	Eggplant	Solarization	131	...	1	1
	Cotton	Solarization	52	...	1	1
	Corn	Solarization	28	...	1	1
Revivim	Tomato	Solarization	17	...	1	1
	Tomato	Methyl bromide	–27	...	1	1
	Sorghum	Solarization	122	...	1	1
	Sorghum	Methyl bromide	40	...	1	1
Sede Eliyyahu	Tomato	Solarization	15	101–130	3	1
Tirat Zevi	Tomato	Solarization	18	16–120	2	1
Yad Mordekhay	Tomato	Solarization	113	...	1	1
	Tomato	Metham-sodium	82	...	1	1
Yahel	Tomato	Solarization	152	137–187	2	2
Zippori	Tomato	Solarization	51	32–170	2	2

<sup>a</sup> Solarization, metham-sodium, and methyl bromide treatments were carried out in the field at the indicated locations. After, soil was collected and used to plant the bioassayed plants in the greenhouse in six replicates. After 28 days of growth, dry weights were determined.

<sup>b</sup> IGR = Increased growth response, calculated as the percentage of increase in shoot dry weight over the untreated control. Midline dots indicate that only one experiment has been carried out.

<sup>c</sup> Total number of experiments at each location between 1984 and 1988. Each experiment represents a different year and is a mean of two repeated greenhouse tests.

<sup>d</sup> Number of experiments (out of total) in which the difference between untreated and disinfested soil was significant according to Student's *t* tests, ( $P \leq 0.05$ ).

exception of sorghum and corn, which were seeded directly and thinned after emergence to five plants per pot, test plants initially were sown in the test soils and transplanted 1 day after emergence to new pots (five plants per pot) filled with the same soil. Plants were grown in the greenhouse (22–28 C) for 28 days without fertilization. They were then uprooted, and roots and the adhering rhizosphere soil were separated from the plant and used for rhizosphere and root-microbial analyses. Shoots were cut, and dry weight (70 C for 48 hr) was determined. Growth response was calculated as increased dry weight over the untreated control (Table 1). Experiments were carried out in a completely randomized design with six replicates for each treatment.

**Field experiments.** Field plots (8 × 16 m) in randomized complete block designs with four replicates were either solarized or left untreated. Tomato or cotton plants were grown in these plots according to standard agricultural recommendations. Five plant samples were collected from each plot at various periods after planting, and dry weight of the shoots was determined. Roots and adhering soil were used for rhizosphere and root-microbial analyses.

**Root-development assay.** Modular narrow glass boxes (20 cm long, 20 cm high, 1.5 cm wide) were filled with Rehovot or Bet HaShitta soils, either untreated or solarized, and moistened to field capacity (Fig. 1). The walls of the boxes could be separated, enabling easy separation of the root system from the soil. Seeds of tomato plants were sown on the surface of the soil and covered with 1 cm of the same soil. Boxes were placed in a completely randomized design in a growth chamber (25 C with 14 hr of daily artificial illumination) with five replicates for each treatment. Root length and microbial populations were assessed during seedling growth, as indicated.

**Microbial assays. Microbial counts by soil dilution.** Three 5-g soil subsamples of each replicate were added individually to 45 ml of sterile water agar (0.1%) supplemented with  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1%), shaken for 15 min on a reciprocal shaker, and then serially diluted with the same solution. Samples of 0.1 ml (for bacterial and actinomycete counts) or 0.2 ml (for fungal counts) were spread on five petri dishes that contained the appropriate selective agar medium. Dishes were incubated in the dark at 28 or 40 C for the determination of thermotolerant microorganisms. Colonies were counted after 4–10 days. Results are expressed as colony-forming units (cfu) per gram of soil (dried at 105 C for 48 hr).

**Microbial assay of rhizosphere.** Plants from greenhouse pots, root-development glass boxes, or experimental field plots were removed from the soil, along with their roots and adhering soil. Soil adhering to the roots was collected by shaking the roots

in sterile tubes. Remaining soil, which tightly adhered to the roots (less than 5% of the total amount of rhizosphere soil), was collected by shaking in sterile 0.1% water agar supplemented with 0.1%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . Both soil fractions were combined, constituting the rhizosphere soil sample. Rhizosphere soil suspensions were serially diluted, spread on the appropriate medium, and incubated as described.

**Microbial assay of roots.** Roots were washed under running tap water, cut into 2- to 3-mm segments, blotted on sterile filter paper, placed on the appropriate agar medium, and incubated as described. Results are expressed as percentage of segments colonized with the indicated microorganism. A direct assay of the total microbial populations of the whole root tissue was carried out by macerating washed roots in 0.1% water agar (supplemented with 0.1%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) with a high speed homogenizer (Ultra Turrax, Janke & Kunkel, Germany) for 1 min. The suspension was diluted further, and 0.1- to 0.2-ml samples from the proper dilution were spread on the solidified media. Microbial populations in the interior root tissues were assayed similarly to the whole root, except washed roots were surface-disinfested with 1% NaOCl for 30 sec before maceration.

**Isolation and inoculation of microorganisms.** Colonies of randomly selected microorganisms isolated from rhizosphere or root tissues were transferred to growth media: N for bacteria and actinomycetes, KB for fluorescent pseudomonads, and PDA for fungi. Cultures were incubated for 48 hr for bacteria and actinomycetes and 8 days for fungi. Bacteria were suspended in distilled water containing 0.5 mM  $\text{CaCl}_2$  (13). Fungal conidia and mycelial fragments were suspended in tap water. Final inoculum density of fungi was  $10^6$  cfu/ml, as determined with the aid of hemacytometer; bacterial concentrations were  $10^8$ – $10^9$  cfu/ml, as determined by optical density. Roots of tomato, 1–2 days after seedling emergence, were dipped for 10 min in the suspension of each bacterial or fungal isolate tested, and then the seedlings were replanted in natural Rehovot soil, which was uninfested with known pathogens. In some experiments, tomato seeds were immersed in suspensions of isolates of fluorescent pseudomonads for 20 min and then sown in untreated Rehovot soil. Isolates of *P. pinophilum* also were tested by mixing washed conidia with soil to a concentration of  $10^6$  cfu/g before planting tomato seedlings. Plants were uprooted 28 days after inoculation, and dry weight of the shoots was determined. Reisolation of microorganisms from interior root tissues of inoculated plants was done by placing 2- to 3-mm surface-disinfested segments of washed roots (1% NaOCl for 30 sec) on the appropriate medium.

**Identification of fluorescent pseudomonads.** Fluorescent pseudomonads were identified by fatty acid and methyl ester compositions with gas chromatographic analysis (24). Identifications were performed in the laboratory of B. C. Hemming, Monsanto Co., St. Louis, MO.

**Test for antagonism on agar.** Nonfluorescent and fluorescent pseudomonad bacteria were cultured on N or KB medium, respectively, for 48 hr. Then, bacteria from each isolate were spotted at three equidistant points 1 cm from the edge of petri dishes (85 cm diameter) containing PDA. After 48 hr of growth in the dark at 30 C, mycelial disks of a test fungus were placed in the center of each dish. After further incubation in the dark at 30 C for 2–3 days, bacterial isolates that induced a fungal inhibition zone were recorded. Test fungi were *Sclerotium rolfsii* Sacc. and *Macrophomina phaseolina* (Tassi) Goidanich.

**Statistical analyses.** Greenhouse experiments, root-development assays, and microbial analyses were conducted at least twice, unless otherwise indicated. Variances between experimental trials were homogeneous, and, thus, data from repeated experiments were pooled. Statistical analyses of the results included analysis of variance, correlation, linear regression, Student's *t* test, or calculations of standard error as indicated. Percentages were transformed to arcsine-square roots before analyses. Hierarchic analyses were done for the inoculation tests to determine whether the origin of the inoculants had a significant effect on plant growth. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC) at  $P \leq 0.05$ .

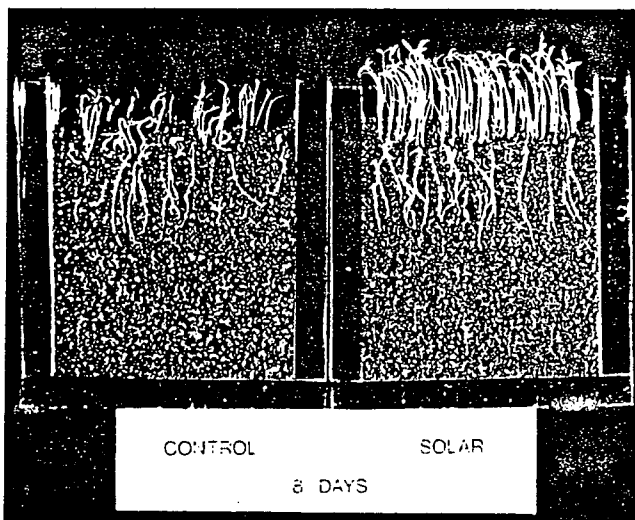


Fig. 1. Effect of soil solarization (solar) on emergence and growth of tomato seedlings in a glass box for root-development assay with Bet HaShitta soil.

## RESULTS

**Assay of plant growth. Greenhouse experiments.** Growth response was determined in soils from 24 locations, with tomato as the major test plant. Compared with untreated controls, a significant increase in plant growth was recorded for most of the tested soils and plants after soil disinfection (Table 1, Fig. 1). In solarized soils, the average percentages of increase in dry weight of tomato ranged from 9 to 152%. In six soils treated with methyl bromide, the average percentages ranged from 20 to 51%, plus one case of decreased growth. Significant increased growth response was recorded in cotton and tomato plants when treated with the simulated heating system. An increase in growth of tomato was evident in at least one experiment at each location. Of 46 experiments in solarized soils and seven in soils treated with methyl bromide, 39 and four, respectively, resulted in a significant growth increase in tomato plants. In one experiment (at Yad Mordekhay), increased growth in tomato also was evident in soil treated with metham-sodium. No visible symptoms of diseases were observed in any of the greenhouse experiments.

Regression analysis showed a significant, inverse relationship between increased growth of tomato in solarized soils and soil pH (Fig. 2), but not with organic matter, clay, silt, or sand content of the soil ( $r^2 \leq 0.25$ ). For example, high increased growth values of 68 and 119% were recorded in two soils that differed greatly in texture (Bet HaShitta [75% clay] and Rehovot [3.8% clay], respectively).

Emergence of eggplant and pepper seedlings in one solarized Rehovot soil occurred 1-2 days earlier than in the comparable untreated soil. Increased growth of tomato plants was observed in soil from Sede Eliyyahu, although an initial growth retardation

of carrot plants in solarized soil occurred in a previous field study carried out in this soil (12).

The duration of the increased growth phenomenon was determined. Untreated and solarized soil samples were collected from the field in Rehovot in October 1987, 1 mo after solarization was terminated. The dry weight of tomato shoots grown in pots filled with the solarized soil was significantly higher (146%) than that of plants grown in untreated soil. The field plots were left undisturbed, except for weeding. Six months later, soil samples again were collected and the plant-growth assay was repeated. A significant increase (114%) in shoot dry weight after soil solarization still was evident.

Mineral nutrient content was recorded in 13 soils. In most cases, solarization resulted in an increase in mineral levels, i.e.,  $\text{NO}_3^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{+2}$ , and  $\text{Mg}^{+2}$ , and in electrical conductivity (data not shown) similar to a previous study (5). In all cases, solarization reduced the soil pH by 0.1-0.4 units.

**Increased growth under field conditions.** Growth response of tomato or cotton plants was determined periodically at three field locations (Table 2). Increased growth was recorded for both species (19-100%), but was more pronounced with tomato than with cotton plants. Growth still was evident 70 days after planting.

**Root development in growth-chamber assays.** Enhanced root development of tomato seedlings was recorded in two solarized soils during the first 11 days after sowing in a glass-growth apparatus (Figs. 1 and 3), and was observed even before seedling emergence. Similar results in root-length measurements were obtained in Bet HaShitta soil (results not shown).

**Effect of solarization on microbial populations. Nonrhizosphere soil.** A significant reduction in populations of bacteria and fungi was recorded in most of the soils tested (Tables 3 and 4). Popu-

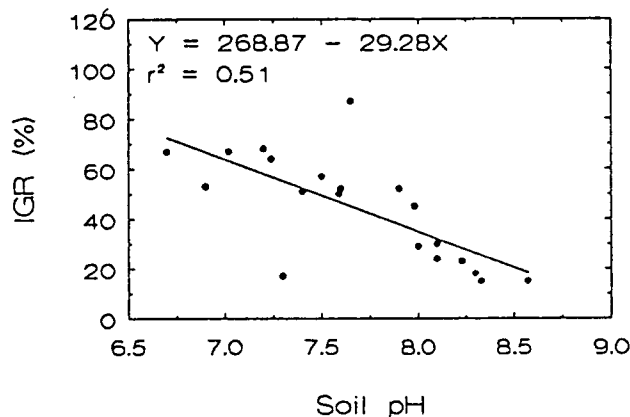


Fig. 2. Relationship between increased growth response (IGR) of tomato plants and pH of various soils. Variation due to regression was significant ( $P = 0.05$ ).

TABLE 2. Effect of solarization on increased growth response (IGR) of plants tested under field conditions

Location	Crop	Planting date	Days after planting	IGR <sup>a</sup> (%)	Significance <sup>b</sup>
Bet HaShitta	Tomato	4/89	21	79	S
			42	45	S
Rehovot	Tomato	5/88	14	80	S
			28	100	S
			70	67	S
			70	17	NS
Eden	Cotton	4/87	14	22	S
			14	19	S
			28	14	NS
			70	17	NS

<sup>a</sup> Calculated as percentage of increase in shoot dry weight over the untreated control.

<sup>b</sup> S and NS represent significant or nonsignificant differences ( $P \leq 0.05$ ) between solarized and unsolarized soil, according to Student's *t* tests.

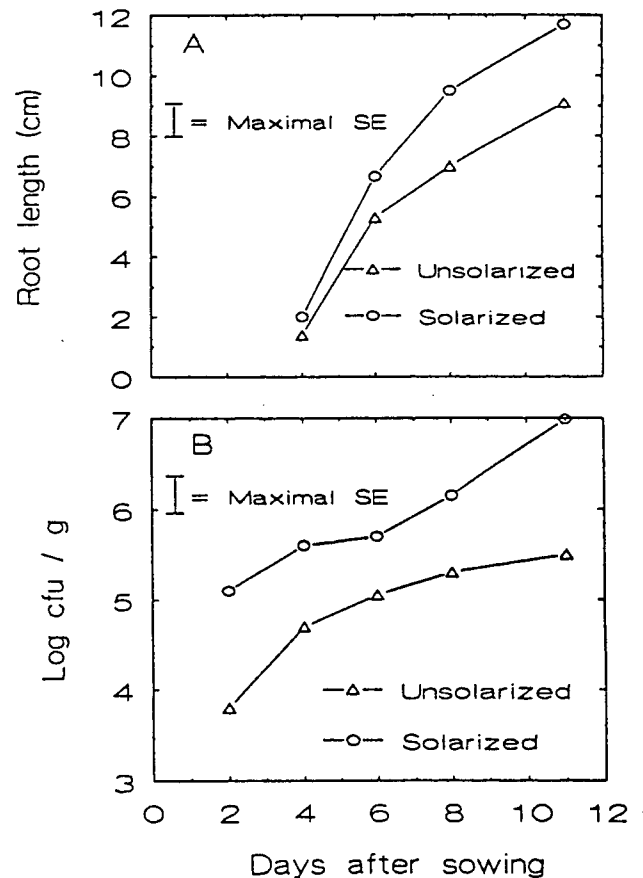


Fig. 3. Effect of solarization (Rehovot soil) on A, length of tomato roots and B, on population densities of fluorescent pseudomonads in the rhizosphere. Test carried out in a glass box for root-development assay. cfu = Colony-forming units per gram of soil.

lations of bacteria and of fungi were reduced 0-93% and 41-97%, respectively, by solarization. Populations of *P. pinophilum*, which was found in seven of 11 tested soils and adversely affected plant growth in preliminary experiments, were considerably reduced (94-99%). In most soils, populations of fluorescent pseudomonads were very low or at undetectable levels in both solarized and unsolarized soils. Changes in microbial populations were recorded at different depths in Rehovot soil (Fig. 4). A general trend of a decrease in populations with increasing soil depth in both solarized and unsolarized soils was observed. Smaller populations of all microorganisms tested were recorded in solarized soil at

all depths, compared with unsolarized soil, thus, indicating an effect of solarization even in deep layers (Fig. 4). At all depths, fluorescent pseudomonads were detectable in the unsolarized soil, but not in the solarized soil. Actinomycetes were the group least affected by solarization to a depth of 60 cm. Populations of thermotolerant bacteria and fungi, i.e., those able to grow at 40 C, also were reduced after solarization, whereas thermotolerant actinomycetes were less affected (Table 4).

**Rhizosphere.** Four weeks after transplanting, bacterial populations in the rhizosphere of tomato plants were much larger in most soils, as compared with the corresponding nonrhizosphere

TABLE 3. Microbial population densities (colony-forming units per gram) in nonrhizosphere and rhizosphere soils, and root colonization of tomato plants as affected by soil solarization

Location	Treatment	Nonrhizosphere soil <sup>a</sup>			Rhizosphere soil				Root colonization (%) <sup>b</sup>		
		Bacteria ( $\times 10^6$ )	Fungi ( $\times 10^3$ )	<i>Penicillium</i> <i>pinophilum</i> ( $\times 10^3$ )	Bacteria ( $\times 10^6$ )	FP <sup>c</sup> ( $\times 10^4$ )	Fungi ( $\times 10^3$ )	<i>P.</i> <i>pinophilum</i> ( $\times 10^3$ )	FP	<i>Pythium</i>	<i>P.</i> <i>pinophilum</i>
Barqay	Control	100* <sup>d</sup>	31*	0 <sup>e</sup>	3,200	1*	93*	0	7*	0	0
	Solarization	50	5	9	2,000	20	6	0	30	0	0
Bet Dagan	Control	9*	110*	80*	16	1*	77*	30*	3*	32*	0
	Solarization	2	41	3	16	25	6	0	30	0	0
Bet Hananya	Control	150*	66*	26*	186*	1	61	10*	46*	66*	0
	Solarization	11	10	0	82	1	58	1	78	0	0
Bet HaShitta	Control	70*	27*	23*	150*	11*	44*	63*	56	0	21*
	Solarization	9	4	0	85	106	21	0	68	0	0
Bsor	Control	22*	44*	0	270	6*	45*	0	12*	60*	16*
	Solarization	9	8	0	280	80	7	0	51	0	4
Gilgal	Control	61*	32*	0	170*	2*	20*	15*	4*	0	0
	Solarization	12	4	0	16	30	3	1	51	0	0
Maale Gilboa	Control	13	51*	16*	110	1*	63	0	17*	40*	3
	Solarization	13	30	0	93	13	61	0	52	0	1
Sede Eliyyahu	Control	120*	46*	40*	840*	1*	50*	0	22	24*	11*
	Solarization	17	5	0	280	130	25	0	34	0	0
Yahel	Control	79*	31*	30*	140	1*	33*	0	8*	20*	20*
	Solarization	7	8	0	130	46	15	0	88	0	0
Zippori	Control	22*	53*	0	240*	1*	48*	10*	25*	76*	23*
	Solarization	2	6	0	24	60	7	0	67	0	0

<sup>a</sup> Composite sample of a soil collected after termination of solarization.

<sup>b</sup> Percentage of root segments colonized by the indicated microorganism.

<sup>c</sup> FP = Fluorescent pseudomonads.

<sup>d</sup> Asterisk denotes a significant difference ( $P \leq 0.05$ ) from the corresponding solar treatment, according to Student's *t* tests.

<sup>e</sup> Zero denotes below detectable level.

TABLE 4. Microbial population densities (colony-forming units per gram) in Rehovot soil and in different parts of tomato roots as affected by solarization or by heating in a simulation system after incubation at the indicated temperatures

Organism	Temperature <sup>a</sup> (C)	Nonrhizosphere soil <sup>b</sup>			Rhizosphere soil			Whole root tissue <sup>c</sup>		Interior root tissue <sup>d</sup>		Root colonization (%) <sup>e</sup>	
		Unsolar.	Solar.	Simul.	Unsolar.	Solar.	Simul.	Unsolar.	Solar.	Unsolar.	Solar.	Unsolar.	Solar.
Bacteria ( $\times 10^6$ )	30	137	18* <sup>f</sup>	15*	3,000	1,200	1,100*	600	450	...	...	...	...
	40	60	12*	...	80	100	...	...	...	...	...	...	...
Fluorescent pseudomonads ( $\times 10^4$ )	30	0 <sup>h</sup>	0	0	15	800*	200*	1.3	200*	0.14	2.8*	18	93*
	40	0	0	...	0	0	...	...	...	...	...	...	...
Actinomycetes ( $\times 10^3$ )	30	12.2	11.3	...	13	11	...	0.36	11*	...	...	...	...
	40	9.8	8	...	10	9.8	...	...	...	...	...	...	...
Fungi ( $\times 10^3$ )	30	36	1.3*	2.4*	340	43*	40*	470	51*	...	...	...	...
	40	11	0.54*	...	48	30	...	...	...	...	...	...	...
<i>Penicillium</i> <i>pinophilum</i>	30	60	0*	0*	2,500	0*	0*	500	0*	...	...	38	1*
<i>Fusarium</i> spp.	30	720	0*	0*	3,500	500*	350*	500	200*	...	...	66	67
<i>Pythium</i> spp.	30	...	...	...	...	...	...	...	...	...	...	65	0*

<sup>a</sup> Temperature of incubation of the indicated organisms.

<sup>b</sup> A composite sample of a soil collected after the termination of solarization. Unsolar. = unsolarized soil; Solar. = solarized soil; Simul. = soil heated in a simulation system.

<sup>c</sup> Macerated and washed root tissue.

<sup>d</sup> Disinfested and macerated root tissue.

<sup>e</sup> Percentage of root segments colonized by indicated microorganism.

<sup>f</sup> Asterisk denotes a significant difference ( $P \leq 0.05$ ) from the corresponding unsolarized treatment according to Student's *t* tests.

<sup>h</sup> Midline dots indicate that this parameter was not tested.

<sup>h</sup> Zero denotes below detectable level.

soils (Tables 3 and 4). This trend was less pronounced with fungi. An increase in populations of fluorescent pseudomonads in the rhizosphere of plants in the solarized soils of up to 130-fold higher than in the comparable unsolarized soils was recorded. This rapid establishment of fluorescent pseudomonads was evident at early stages of seed germination, reached its highest level at seedling emergence, and remained high throughout the growth period (Figs. 3 and 5). Similarly, solarization increased populations of fluorescent pseudomonads in the rhizosphere of emerging tomato seedlings grown in soils collected from Magen, Eden, and Sede Eliyyahu soils (data not shown). Regression analysis showed significant, inverse relationships between soil pH levels and the population densities of fluorescent pseudomonads in unsolarized soil ( $r^2 = 0.36$ ; equation of regression line:  $\log$  bacteria number =  $51.3 - 6.17 \text{ pH}$ ) or in solarized soil ( $r^2 = 0.38$ ; equation of regression line:  $\log$  bacteria number =  $214.2 - 29.7 \text{ pH}$ ). No significant regression was found between pH level and populations of fungi ( $r^2 < 0.36$ ) (data not shown). Recovery of *P. pinophilum* remained low in the rhizosphere of plants in solarized soil. Populations of actinomycetes were not greatly affected by solarization (Table 4).

**Roots.** The percentage of colonization of roots of tomato plants grown in the solarized soils by fluorescent pseudomonads was higher than in corresponding unsolarized soils (Tables 3 and 4). This was also true for populations of fluorescent pseudomonads in whole and interior root tissues, as differentiated by surface-disinfestation. In contrast, colonization of roots by *Pythium* spp.

and *P. pinophilum* was reduced to very low or undetectable levels in the solarized soils. Plant roots in solarized soils were colonized intensively by actinomycetes (Table 4). Similar microbial trends were recorded when assessing colonization of root segments or

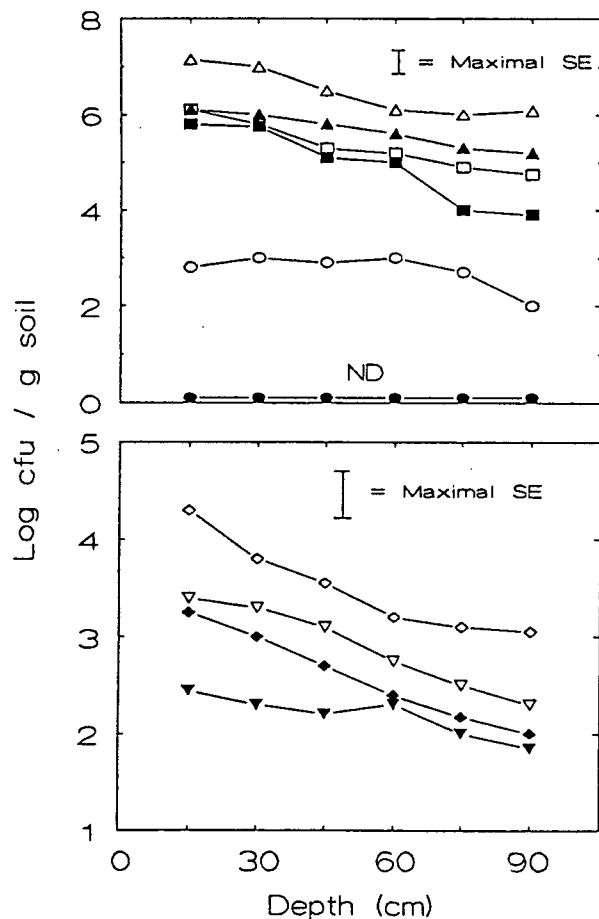


Fig. 4. Effect of soil solarization (Rehovot soil) on microbial population densities in the 15- to 90-cm depth.  $\Delta$ ,  $\blacktriangle$  = bacteria;  $\square$ ,  $\blacksquare$  = actinomycetes;  $\diamond$ ,  $\blacklozenge$  = fluorescent pseudomonads;  $\nabla$ ,  $\blacktriangledown$  = *Fusarium* spp. Open symbols represent untreated soil; black symbols represent solarized soil. ND = below detectable level; cfu = Colony-forming units per gram of soil.

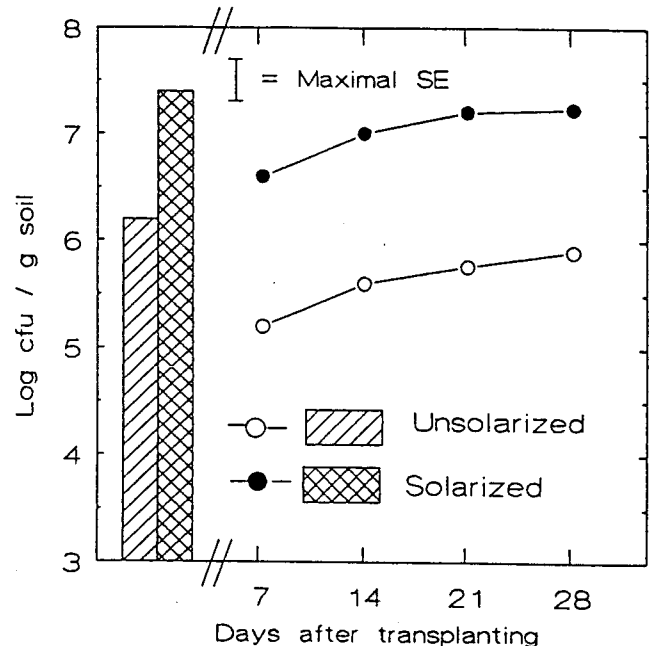


Fig. 5. Effect of solarization (Rehovot soil) on population densities of fluorescent pseudomonads (FP) in the rhizosphere of tomato plants over time. Columns represent FP population densities in the rhizosphere of emerging seedlings before transplanting (10 days after sowing); cfu = Colony-forming units per gram of soil.

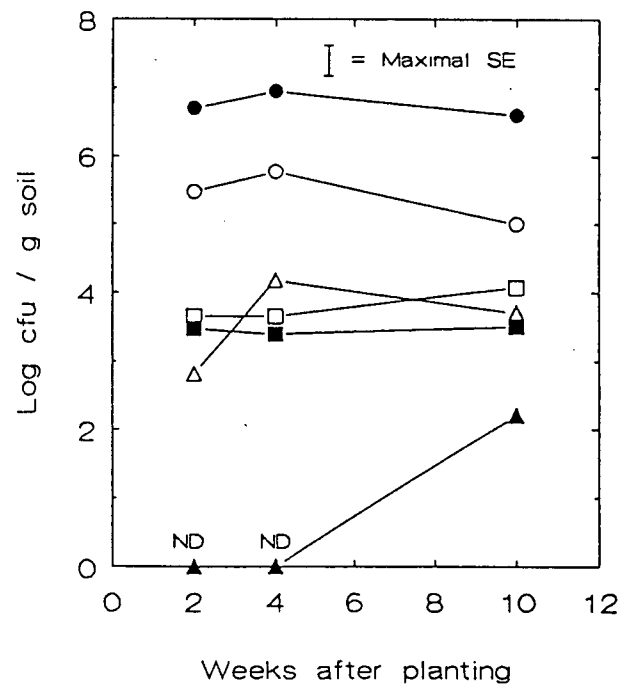


Fig. 6. Effect of solarization on microbial population densities in the rhizosphere of tomato plants grown in the field in Rehovot soil.  $\circ$ ,  $\bullet$  = fluorescent pseudomonads;  $\Delta$ ,  $\blacktriangle$  = *Penicillium pinophilum*;  $\square$ ,  $\blacksquare$  = *Fusarium* spp. Open symbols represent untreated soil; black symbols represent solarized soil. ND = below detectable level; cfu = Colony-forming units per gram of soil.

by directly assessing microbial populations in macerated roots (Table 4).

**Simulated heating.** Tomato plants were grown in untreated and artificially heated Rehovot soil. Changes in populations of total bacteria, fluorescent pseudomonads, total fungi, *P. pinophilum* and *Fusarium* spp. in nonrhizosphere and rhizosphere soils caused by artificial heating were similar to those caused by solarization (Table 4).

**Field conditions.** Samples of rhizosphere soils and roots of tomato or cotton plants were collected periodically from two field experiments. In Rehovot soil, populations of fluorescent pseudomonads in the rhizosphere were increased after solarization during all sampling periods (Fig. 6). Similarly, colonization of tomato roots by fluorescent pseudomonads in the solarized soil to a depth of 60 cm was greater than in the unsolarized soil, whereas colonization by *P. pinophilum* and *Pythium* spp. was suppressed (Fig. 7). Populations of *Fusarium* spp. were not affected by solarization. These trends also were observed with cotton plants in Rehovot soil and with tomato plants in Bet HaShitta soil (results not shown). For example, solarization increased populations of fluorescent pseudomonads in the rhizosphere by 40- and 19-fold at Rehovot and Bet Hashitta, respectively. In both soils, solarization reduced root colonization by *P. pinophilum* and *Pythium* spp. by 80-100%.

**Effect of soil microorganisms on plant growth. Bacteria.** One hundred randomly selected isolates of bacteria from the rhizosphere of tomato plants from unsolarized or solarized Rehovot soil were used to inoculate tomato seedlings. None of these 200 isolates had a significant effect on tomato growth, as compared with uninoculated plants. Fifty isolates of fluorescent pseudomonads from the tomato rhizosphere and 100 isolates from the plant roots from either unsolarized or solarized soils were used to inoculate tomato seedlings. In contrast to the results with non-fluorescent bacteria isolates, three and seven isolates of fluorescent pseudomonads from the rhizosphere and 10 and 29 isolates from the roots of plants from unsolarized and solarized soils, respectively, significantly increased the dry weight of tomato shoots by 25-65% over the uninoculated plants. Plant growth increase also was evident when the seed inoculation method was used with 15 of these isolates. Twelve isolates of fluorescent pseudomonads, which significantly increased dry weight, were identified by fatty-acid composition with chromatographic analysis. Six isolates were identified as *P. putida*, three as *P. fluorescens*, and three as *P. alcaligenes*, with similarity indices of 0.55-0.72, 0.45-0.68, and 0.40-0.48, respectively.

**Fungi.** One hundred randomly selected fungal isolates from soil in the rhizosphere and 100 isolates from tomato roots, from either unsolarized or solarized Rehovot soil, were tested. Of the fungi from the rhizosphere, nine isolates from unsolarized and three from solarized soil significantly reduced plant growth by 19-40%. Of the fungi from roots, 23 isolates from unsolarized and nine from solarized soil significantly reduced plant growth by 18-42%. Most of the growth-suppressing fungi were identified as *P. pinophilum*. Of the 50 isolates of *P. pinophilum* randomly isolated from tomato roots from unsolarized Rehovot or Bet HaShitta soil, 12 and 9 isolates, respectively, significantly reduced growth of inoculated tomato seedlings by 32-41% and 42-57%. Twelve growth-suppressing isolates of *P. pinophilum* that originated from Bet HaShitta soil also were tested by soil infestation instead of root dipping. In all cases, significant plant growth suppression resulted. The growth-suppressing fungi could be reisolated (after surface-disinfestation) from the interior root tissues, indicating the invasion and establishment of these fungi in the roots. None of the fungi tested stimulated plant growth.

Inoculation tests were repeated with representative isolates of fluorescent pseudomonads and *P. pinophilum*. The results were similar to those mentioned.

Hierarchical statistical analyses were conducted to examine the effect of microbial populations on plant growth, with regard to the origin of the population. An analysis of all isolates of fluorescent pseudomonads showed that those originating from plants grown in solarized soil increased plant growth significantly more

than those from plants in unsolarized soil. Similarly, fungi from unsolarized soil decreased plant growth significantly more than those from plants grown in solarized soil.

**Actinomycetes.** One hundred isolates of actinomycetes from the rhizosphere soil of tomato plants of each unsolarized and solarized Rehovot soil were used to inoculate tomato seedlings. None of these isolates significantly affected tomato growth.

**Antagonism on agar.** Isolates of bacteria from the soil, rhizosphere, and roots of tomato plants grown in either solarized or unsolarized soils were tested for antagonistic activity against *S. rolfii* and *M. phaseolina* on agar. With each of the 10 tested combinations, the number of bacteria from solarized soil evincing antagonistic activity was higher than that of microorganisms from the respective unsolarized soil (Table 5).

## DISCUSSION

Increased growth in heat-treated and fumigated soils (19,34) and in solarized soils (1,5,15,25,31-33) has been extensively reported. We also demonstrated increased growth of several crops in several solarized or fumigated soils in the greenhouse (Table 1). Increased growth and the respective microbial changes also were evident in three field experiments (Table 3; Figs. 6 and 7). Results in soils artificially heated to simulate solarization were similar to those obtained in solarized soil (Tables 1 and 4), thus, supporting the reliability of this simulated system (9,36). Suppression of plant growth by solarization was not observed in this study, but has been reported (12). Reproducibility of increased growth response in solarized soils was evident when the soil was solarized in different years at the same location, e.g., at Barqay, Bet Dagan, and Rehovot (Table 1).

Increases in crop yield after solarization were obtained in field experiments without apparent infestation (8,10,25). However, the relationship between increased crop yield in the field and increased growth of young plants in greenhouse experiments has to be studied further because of important economic implications.

Previous investigators correlated increased growth response with chemical changes in the soil (2,5,20,33); the increase in

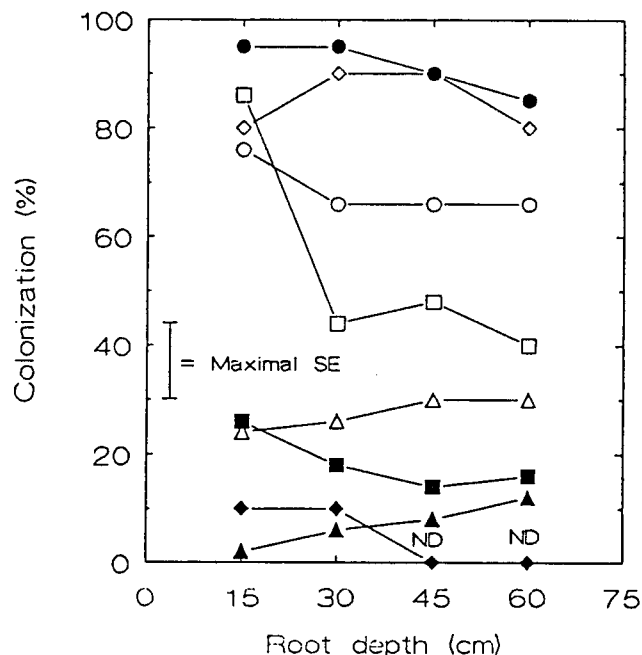


Fig. 7. Effect of solarization on microbial colonization at various depths of roots of tomato plants grown in the field in Rehovot soil. Colonization was calculated as percentage of segments colonized with the indicated organism. ○, ● = fluorescent pseudomonads; △, ▲ = *Penicillium pinophilum*; □, ■ = *Fusarium* spp.; ◇, ◆ = *Pythium* spp. Open symbols represent untreated soil; black symbols represent solarized soil.

TABLE 5. Antagonistic activity of fluorescent pseudomonads or nonfluorescent bacteria against *Sclerotium rolfsii* and *Macrophomina phaseolina* in culture

Source of soil	Bacteria origin	Soil treatment <sup>a</sup>	Micro-organism <sup>b</sup>	No. of isolates	<i>S. rolfsii</i> <sup>c</sup>	<i>M. phaseolina</i> <sup>c</sup>
Kfar Warburg	Rhizosphere	US	NFC	60	19	15
	Rhizosphere	S	NFC	60	20	21
Rehovot	Soil	US	NFC	60	6	5
	Soil	S	NFC	60	7	6
	Rhizosphere	US	NFC	60	3	7
	Rhizosphere	S	NFC	60	11 <sup>d</sup>	10
	Root	US	NFC	60	7	19
	Root	S	NFC	60	16 <sup>*</sup>	23
	Root	US	FP	45	1	9
	Root	S	FP	45	4	12

<sup>a</sup> US = Unsolarized soil; S = solarized soil.

<sup>b</sup> NFC = Nonfluorescent bacteria; FP = fluorescent pseudomonads.

<sup>c</sup> Number of isolates that inhibited growth. The total numbers of isolates from unsolarized Rehovot soil that inhibited growth of *S. rolfsii* and *M. phaseolina* were 17 and 40, respectively and the total numbers of isolates from solarized Rehovot soil were 38 and 51, respectively. The total number of isolates used was 225 each from unsolarized and solarized Rehovot soil.

<sup>d</sup> Asterisk denotes a significant difference ( $P = 0.05$ ) between isolates from solarized and unsolarized soil from Rehovot, according to Student's *t* tests.

mineral nutrients in solarized soils we obtained in our study apparently may have a role in the phenomenon. However, the long-lasting effects of the increased growth observed in this study and by others (1,16,25,31) may be due to shifts in the components of the soil microbial populations.

Significant regressions between soil pH and increased plant growth (Fig. 2) or populations of fluorescent pseudomonads were shown, yet it is not known how soil pH affects factors related to the increased growth, or what the relationship is between pH levels in the soil and in the rhizosphere. Correlations between soil properties (e.g., pH) and increased plant growth and microbial colonization of the rhizosphere might be useful for developing systems for predicting increased plant growth in soils.

Populations of bacteria and fungi (including thermotolerant fungi) were reduced by solarization. Actinomycetes were least affected, as also reported by others (14,21,31). Stapleton and DeVay (31) found a decrease in populations of various bacteria (including fluorescent pseudomonads) and fungi in the upper soil layer immediately after solarization; whereas, in other studies bacteria increased after solarization (14,21). Microbial changes in solarized soils were observed even at depths between 60 and 90 cm (Figs. 4 and 7), at which the temperature is not increased markedly (15). Thus, factors other than heat, such as volatiles, should also be considered.

Significant changes in microbial populations, especially fluorescent pseudomonads, occurred in the root zone. Populations of these bacteria were very low in nonrhizosphere soil and decreased further after solarization. However, they rapidly colonized the rhizosphere and plant roots in the solarized soil, and their populations increased up to 130-fold over numbers in unsolarized soil. This trend was evident in 11 different soils, including field experiments (Tables 3 and 4; Figs. 3, 5-7). Surprisingly, heat-sensitive fluorescent pseudomonads, rather than thermotolerant microorganisms, were the most successful in exploiting the rhizosphere. Apparently, heat sensitivity of these bacteria is counterbalanced by other beneficial characters, such as short generation time and probably rapid growth as compared with other colonizers. Being poor competitors (22), fluorescent pseudomonads eventually may benefit from the reduction in population densities of competing microorganisms by solarization. Root exudates are the dominant factors in the rhizosphere (6) and may be the trigger or signal for the massive proliferation of these bacteria in this region, as verified by us in another study (A. Gamliel and J. Katan, unpublished data). Of the bacteria tested in this study, only fluorescent pseudomonads significantly increased growth of inoculated plants. Fluorescent pseudomonads have been studied intensively as plant growth-promoting rhizobacteria (17,18,27). Hence, elucidating the mechanisms related to their successful establishment in solarized soils has broad implications. Isolates of *Bacillus* and *Trichoderma* spp. also im-

prove plant growth (3,4). Because these organisms are stimulated by solarization in certain cases (16,32), their possible role in increased plant growth should be considered in future studies. Root colonization was assessed by using root-segment and root-maceration methods (Tables 3 and 4). The segment-colonization assay is less sensitive but is much less tedious and time-consuming than the direct assay.

With the increase in growth-promoting fluorescent pseudomonads, a decrease in population densities of *P. pinophilum*, which suppresses plant growth, was observed. The two microbial shifts may have contributed simultaneously to increased plant growth in solarized soil in our study. The harmful effect of *Penicillium* spp. was reviewed by Salt (23). *P. pinophilum* fulfills some of the requirements for a "minor pathogen" (23): it suppresses plant growth although distinctive disease symptoms are lacking; it parasitizes root tissues; it is widely distributed in soils (Tables 3 and 4); and it is not restricted to one host (unpublished data). The possibility that the root-surface microflora also include deleterious microorganisms, namely, those affecting the plant by metabolites without parasitizing its tissues (23,35), was not excluded. Relationships between control of *Pythium* spp., known to affect root growth adversely (29), and increased growth response has yet to be determined. A possible beneficial effect of fluorescent pseudomonads is the suppression of deleterious and other harmful microorganisms through aggressive colonization of roots and antibiotic production (17,18,26). A similar mechanism may operate in solarized soils. An increase in population densities of bacteria and fungi showing antagonistic activity in culture may indicate the potential of such microorganisms in biological control. The poor establishment of *P. pinophilum* and *Pythium* spp. in the solarized soils might be attributable to induced suppressiveness, as shown with certain pathogens (9). It is not yet known whether microbial shifts related to increased growth response in solarized soils are related to mechanisms of induced suppressiveness (9,15).

By using hierarchic analyses, we demonstrated qualitative changes in population densities of beneficial bacteria and of harmful fungi in the plant root zone in solarized soils. We suggest that increased plant growth can be attributed to a combination of biotic and abiotic mechanisms, which may vary in different soils. The involvement of other biotic and abiotic mechanisms related to plant growth, e.g., mycorrhizae, phytotoxic-decomposition products, and plant growth-promoting substances (3,4,16) should not be excluded. A fuller understanding of the mechanisms of the increased growth response may contribute to knowledge of the basic principles of plant health.

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# 9. Phytotoxic effects of combining heating with fertilizers.

The solarized trees in Mazor showed severe symptoms of chlorosis. This phenomenon was not observed in other locations. We examined the possibility that this is due to the fact that the trees were fertilized with ammonium nitrate during solarization. Three year old apple plants were either solarized or nonsolarized and amended or non-amended with ammonium nitrate. Chlorosis intensity was evaluated. Results (Fig. 15) show that such a combination was harmful to the plants.

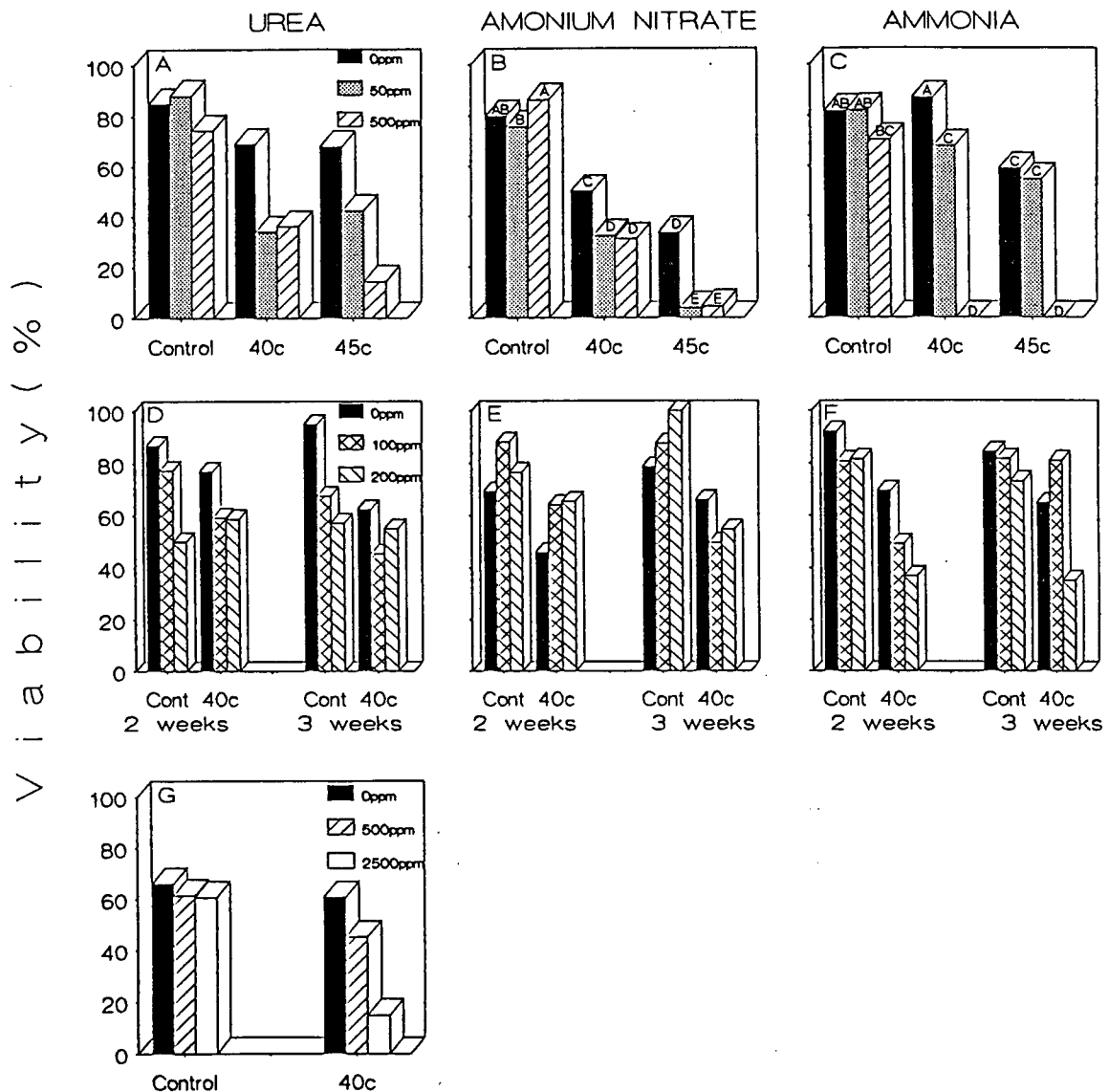


Fig. 13. Effect of heating at two temperatures and nitrogenous compounds on viability of sclerotia of *Sclerotium rolfii*.

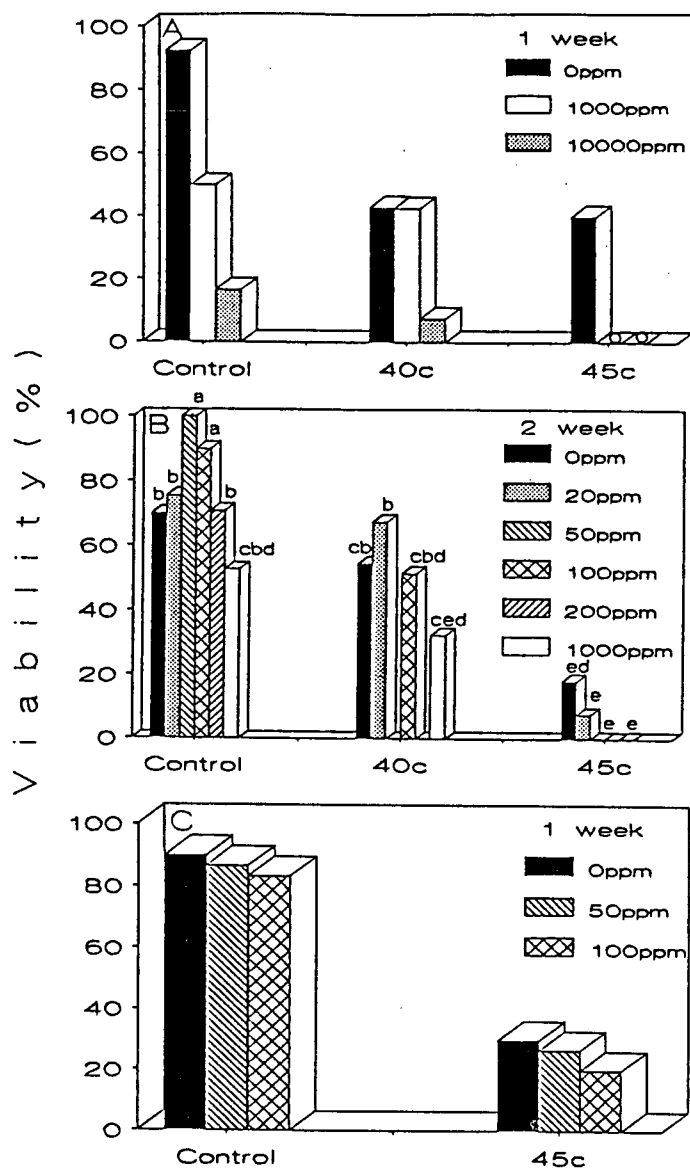


Fig. 14. Effect of heating at two temperatures and cabbage residues at 50-10,000 ppm on viability of sclerotia of *Sclerotium rolfii*.

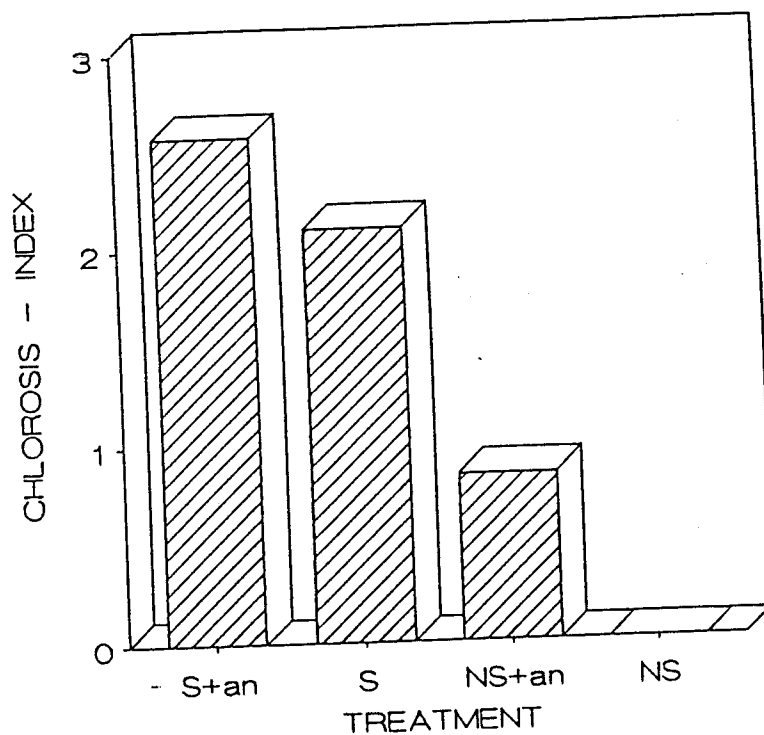


Fig. 15. Effect of soil solarization and ammonium nitrate on chlorosis intensity in apple plants. S = Solarized; NS = nonsolarized; an = ammonium nitrate.

II. STUDIES IN THE USA

BARD -- FINAL TECHNICAL REPORT

BARD Proposal No. I-1291-87

October 1, 1988 to September 30, 1992

James E. DeVay, Department of Plant Pathology  
University of California, Davis, CA 95616 USA

**TITLE**

Postplant control of soilborne diseases of fruit tree crops by soil solarization.

**ABSTRACT**

Bare root second leaf apricot (*Prunus armeniaca*) and almond (*P. dulcis*) trees were planted in soil infested with *Verticillium dahliae* and solarized with clear or black polyethylene film. The incidence of foliar wilt symptoms and vascular discoloration due to *Verticillium* wilt disease were reduced 86% to 100% in both apricot and almond trees the following season by both clear and black films. Trees solarized from the time of planting with clear film did not survive or grow as well as those solarized with black film or those in nonsolarized soil. Black film gave better overall results than solarization with clear film, since the intermediate soil temperatures that resulted did not injure the roots as judged by tree survival and annual growth of trunk diameters. Moreover, the prolonged period of soil heating under black film provided control of *Verticillium* wilt equivalent to that obtained with clear film. Significant conservation of irrigation water was achieved by postplant solarization since no additional water was needed by solarized trees for approximately four months following planting, a main consideration in the establishment of new orchards or replant trees in warm, arid climates.

In another study, the heat sensitivity and the effect of soil solarization on the survival of hyphae and spores of three major soilborne pathogens, *Phytophthora cinnamomi*, *P. cactorum*, and *P. megasperma* (high and low temperature isolates) were determined. Viable chlamydospores of *P. cinnamomi* were not detected in infested soil exposed to 45 C for 20 min whereas oospores of a high temperature isolate of *P. megasperma* survived exposure for 30 min at 45 C. An isolate of *P. cactorum* was killed within 30 min at 45 C. The heat sensitivity of the isolates of *Phytophthora* in laboratory experiments closely reflected their inactivation in solarized soil. In field studies, solarized soil reached a maximum temperature of 45 C at the 15 cm depth and 33 C at the 45 cm depth, compared with nonsolarized soil where maximum temperatures of 31 and 28 C were recorded at 15 and 45 cm depths, respectively. No viable spores of *P. cinnamomi* were detected in infested soil after

2 wk at the 30 cm depth or after 4 wk at the 45 cm depth in solarized soil, whereas there was little or no reduction in survival in nonsolarized soil. *P. cactorum* was killed within 2 wk at the 15 cm depth but withstood the effects of soil solarization at the 30 to 45 cm depths. *P. megasperma* was the least heat sensitive of the isolates used, but soil solarization for 4 wk greatly reduced the number of its viable propagules.

#### OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL AND MAJOR RESULTS

The research objectives of this project conducted at the University of California, Davis, were as follows:

1. To study the effectiveness of solarization in soilborne pathogen control in existing orchards as a postplant treatment.
2. To study the effect of solarization on the roots of the solarized trees as affected by the heat, the conservation of soil moisture, and by gas changes in the soil.
3. Mechanism of solarization in existing orchards: to study reinfestation by pathogens, gasses accumulating in soil, and the temperature regimes in the solarized orchard with various degrees of shading.

The following studies addressed Objectives 1 and 2:

- A) Establishment of apricot and almond trees using soil mulching with clear and black polyethylene film: Effects on *Verticillium* wilt and tree health (Appendix I).

#### Material and Methods

Bareroot apricot (*P. armeniaca* cv. Patterson on apricot rootstock) and almond (*P. dulcis* cv. Carmel on 'Nemaguard' peach rootstock) trees were transplanted in a randomized complete block design into Panoche clay loam soil at the University of California West Side Field Station near Five Points in the San Joaquin Valley, in March 1990. The scion and rootstock combinations were selected for maximum susceptibility to *Verticillium* wilt. Trees were planted 2 m apart, with 5 m between rows. Soil was sampled and dry-plated on selective agar medium to determine initial numbers of *V. dahliae*. Previous soil assays at this location indicated the presence of a mixture of cotton-defoliating and nondefoliating pathotypes of *V. dahliae*. Soil treatments were solarized with UV-stabilized, 0.1 mm-thick, clear or black polyethylene film (Ethyl-Visqueen, Inc., Fremont, CA); or not solarized, with 5 to 7 replications of 5 or 6 trees of each of the two species. Trees were planted and flood-irrigated, then solarized two weeks later by fitting polyethylene film strips (3 m wide) over the tree rows and securely burying the edges of the film with soil. Holes in the film were made to slip over the bareroot trees. The film was sealed around the base of

trees with silver duct tape. Film mulches extended ca. 2 m past the end trees of each replication. Soil temperatures were continuously recorded at 18 and 30 cm depths by thermistors connected to a Campbell CR-21 micrologger (Campbell Scientific Inc., Logan, Utah, USA). Nonsolarized control trees were irrigated by a surface drip system on a ca. biweekly schedule (100-125 l/tree/irrigation) throughout the season, while solarized trees received no further irrigation until they showed signs of water stress in August. Film mulches were then removed after 19 wk duration, and trees were drip-irrigated as per the controls for the remainder of the 1990 season, and the entire 1991 season. Soil samples for enumeration of *V. dahliae* after treatment were collected on August 31. Aliquots from soil (ca. 0-25 cm depth) were taken by shovel near the base of each experimental tree, bulked by replication, and dry-plated on selective medium as previously described.

Assessments of tree survival were made during 1990-91, ca. 3, 4, 5, 6, and 18 mo after the clear and black films were applied. Visual determination of incidence of foliar wilt symptoms were made throughout the 1991 growing season, and the pathogen was re-isolated from symptomatic tissue. Incidence of vascular discoloration was determined by destructive sampling of trees at the end of the 1991 season. Tree trunks were sawed off ca. 30 cm above the graft union and major scaffolds were cut off the same distance above their junction with the trunk, then rated for presence or absence of vascular discoloration. Data from each tree species were analyzed separately. Data expressed in percent were arcsin-transformed prior to analysis of variance and mean separation.

## Results

Soil temperatures during the treatment period reached 46, 41, and 33°C at 18 cm depth, and 41, 37, and 32°C at 30 cm depth under clear film, black film, and no film, respectively. Mean diurnal soil temperatures occurring at both depths between June 6 and July 30 are shown (Fig. 1).

Pre-treatment soil assay showed a mean of 10.0 propagules of *V. dahliae* in the 0-30 cm depth range. The pathogen was not detected below 30 cm. Post-treatment soil assay for *V. dahliae* showed that on August 31, propagule numbers in the 0-23 cm depth range near almond trees were 76-82% lower than the control after black or clear film mulching, respectively; and 40-67% lower near the apricot trees (Table 1).

## Tree survival and growth

Under the relatively hot air temperatures occurring during the growing season, trees solarized with clear polyethylene film were severely stressed. By June 6, 76% of the clear-mulched almond

trees and 22% of the apricot trees had collapsed. The mulches were removed on August 9, when surviving trees began to show leaf flagging, and all trees were uniformly drip-irrigated until the end of the season. Nonsolarized trees received 12 irrigations during the season, including the pre-irrigation, totalling ca. 2,344 liter water/tree. In comparison, solarized trees received 6 irrigations, totalling ca. 1,840 l water/tree, a reduction of more than 21% by volume.

At the time of film removal, 91% of the almond trees and 39% of the apricot trees solarized with clear polyethylene were dead. At the same time, only 3% and 6% of the black-mulched almond and apricot trees, respectively, were dead - statistically no different than the nonsolarized control. By the end of the following (1991) growing season, only 3% of almond and 35% of apricot trees solarized with clear film survived. There were no differences, however, in survival between nonsolarized trees and trees solarized with black film (Fig. 2).

At the end of the 1990 growing season, trunk diameters of apricot trees grown under both solarization treatments were significantly smaller than those not solarized; while only the clear plastic film adversely affected almond trunk diameter. After the 1991 season, only the apricot and almond trees solarized with clear film were smaller than the nonsolarized control trees (Figure 3).

### **Verticillium wilt**

Typical foliar symptoms of *Verticillium* wilt became apparent in some of the experimental trees in April, 1991. The 1991 growing season was relatively cool, and symptoms atypically continued to develop throughout the summer; *V. dahliae* was isolated from infected shoots as late as September. Experimental trees were given a final rating for incidence of foliar symptoms in September, then destructively sampled for incidence of vascular discoloration in trunks and primary scaffolds. At that time, 36% of nonsolarized apricot trees showed foliar symptoms, but 92% and 96% had vascular discoloration of scaffolds and trunks, respectively. None of the solarized trees showed foliar symptoms, although up to 25% and 45% had vascular discoloration in scaffolds and trunks (Table 1). By comparison, 50% of nonmulched almond trees showed foliar symptoms, but 68% and 73% had vascular discoloration in scaffold and trunks. Only 7% of trees solarized with black film had foliar symptoms and 10% and 18% had vascular discoloration in scaffolds and trunks (Table 1). Symptom data from almond trees solarized with clear film are not given, since only 1 of the 34 original trees survived.

### **Discussion**

Under the very warm summer climatic conditions of these experiments, solarization with clear polyethylene film was unsuitable, although trees under clear polyethylene film in cooler



areas of the San Joaquin and Sacramento valleys survived and grew well in previous experiments. Trees grown with black film mulching survived as well as the nonsolarized controls. Trunk diameter of the solarized trees was significantly smaller immediately following the solarization treatment, but caught up to the nonsolarized trees the season after the film was removed.

Results with reduced irrigation in the solarization treatments gave further indication that, even under the hot and arid climatic conditions characteristics of the summer season in the San Joaquin Valley, deciduous fruit and nut trees may be established with no more than a preirrigation and perhaps two or three carefully-timed irrigations later in the season, if necessary. Stress produced by soil heating under the clear film mulch may have overwhelmed the limited root systems of the first-leaf almond, and to a lesser extent apricot trees and caused excessive tree mortality. The apricot trees were more tolerant of the treatment. Both species was able to withstand the more moderate heating produced under the black film.

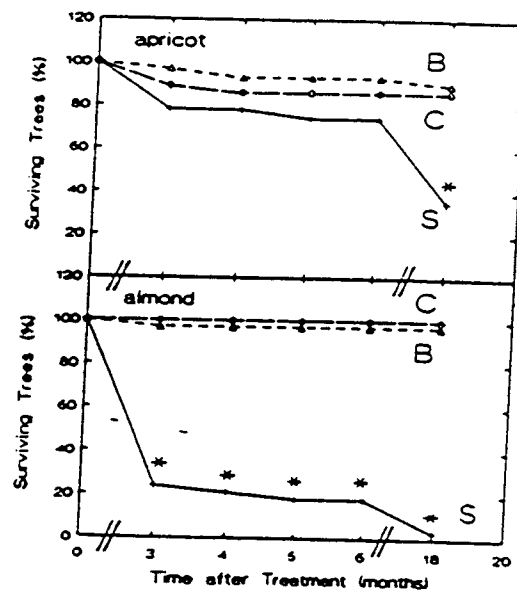
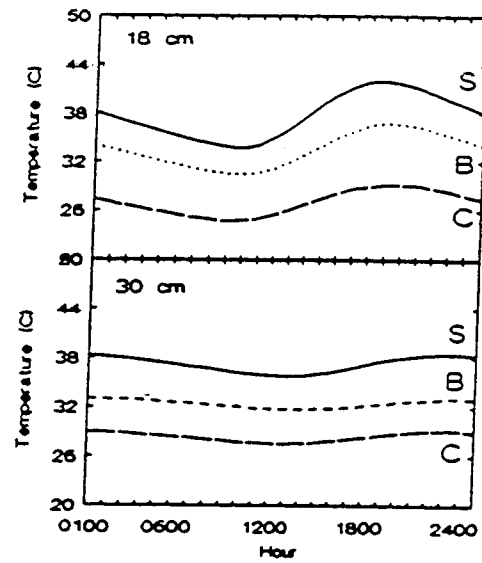
These results showed that solarization with black film was an effective horticultural and pest management practice, controlling *Verticillium* wilt as good as solarization with clear film, while simultaneously conserving irrigation water in a Mediterranean-like climate.

#### Figure Legends

Fig. 1. Mean diurnal soil temperature fluctuation at (A) 18 and (B) 30 cm depth for 54 days (June 6-July 30, 1990) after solarization treatments with clear polyethylene (S), black polyethylene (B) or the nonsolarized control (C).

Fig. 2. Survival of apricot and almond trees during and after soil solarization with clear polyethylene (S), black polyethylene (B), or nonsolarized control (C). Asterisks indicate significantly reduced ( $P < 0.05$ ) survival of the clear film mulch treatment at individual sampling times, according to Duncan's Multiple Range Test.

Fig. 3. Trunk diameter of apricot and almond trees at the end of the first-leaf growing season (1990 - dark bars), and second-leaf growing season (1991 - striped bars). Solarization treatments were removed during the first-leaf season. Control trees received no irrigation from mid-March to mid-August. Different letters indicate significant mean separation at  $P < 0.05$ , according to Duncan's Multiple Range Test. ND = no data, since only one tree remained alive.



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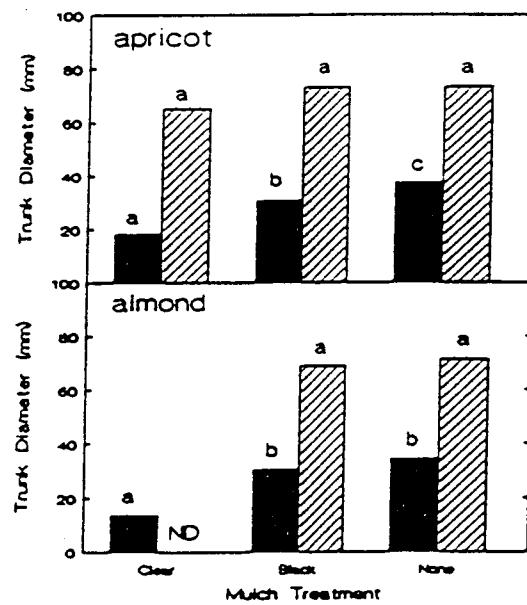


Table 1. Influence of polyethylene film mulching on numbers of Verticillium dahliae in soil and incidence of Verticillium wilt symptoms in second-leaf apricot and almond trees (Five Points, California; 1990-91)<sup>a</sup>

		Incidence of Verticillium Wilt (%)		
Host Species; Soil Mulch Treatment <sup>b</sup>	<u>V. dahliae</u> propagules per g soil <sup>c</sup>	Foliar Symptoms	Vascular Discoloration	
			Primary Scaffolds	Trunk
Apricot <sup>d</sup>				
Clear	1.5 a	0 a	25 a	45 a
Black	1.8 ab	0 a	6 a	34 a
None (Control)	4.5 b	36 b	92 b	96 b
Almond <sup>e</sup>				
Clear	0.8 a	ND <sup>f</sup>	ND	ND
Black	0.6 a	7 a	10 a	18 a
None (Control)	3.4 b	50 b	68 b	73 b

<sup>a</sup> Mulches applied 14 days after trees were planted (March 1990). Data values followed by different letters are different at P<0.05 according to Duncan's Multiple Range Test.

<sup>b</sup> Mulches removed after 19 weeks (August 1990).

<sup>c</sup> Soil sampled in 0-23 cm depth range after mulch removal (August 1990).

<sup>d</sup> 'Patterson' apricot on apricot rootstock.

<sup>e</sup> 'Carmel' almond on 'Nemaguard' peach rootstock.

<sup>f</sup> No data given due to single surviving tree.

B) Thermal sensitivity of three species of *Phytophthora* and the effect of soil solarization on their survival (Appendix II).

### Materials and Methods

Isolates of *Phytophthora* species consisted of an isolate of *P. cinnamomi* from peach, an isolate of *P. cactorum* from apple, and two isolates of *P. megasperma* from peach, including isolates tolerant and sensitive to high temperatures. All isolates were pathogenic on English walnut.

The thermal sensitivity of the *Phytophthora* isolates was tested under controlled environment conditions. Oospores and chlamydospores were mixed with moist soil to give approximately 600 colony forming units per gram of soil. Infested soil (25 cc) was placed in polyethylene bags, which then were partially evacuated to reduce air insulation, and the soil was spread in the bags to make a thin layer (1-2 mm) deep. The bags of soil were then immersed in water at 30, 35, 40, or 45 C for 5, 10, 20 or 30 min. After treatment, the infested soil was assayed using peach leaf disks which were then plated on a selective agar medium. Each treatment was replicated three times. Percent incidence of infected leaf disks from which *Phytophthora* colonies were isolated was used to determine their survival.

The laboratory experiments on thermal sensitivity of the *Phytophthora* isolates were repeated using inoculated walnut twigs. The twigs, approximately 1 cm in diameter and 20 cm long, were inoculated by placing an agar disk with mycelium in a circular bark opening midway between the ends of the twigs. Inoculated twigs were incubated in moist chambers for 5 days at 21 C, during which time cankers ranging from 2 to 4 cm long developed. Twigs were then placed in plastic bags, which were partially evacuated and sealed before immersion in water at 30, 35, 40 or 45 C for 5, 10, 20, or 30 min. To determine the effect of the temperature X time treatments on the survival of isolates of *Phytophthora*, pieces of bark tissue from the margins of the cankers from three replicate twigs were plated on a selective agar medium to determine the percentage of tissue fragments from which the isolated developed.

The sensitivity of the *Phytophthora* isolates to soil solarization was compared in field plot experiments. Inocula consisting of hyphae, oospores or chlamydospores prepared using a colonized oat-vermiculite mixture with V8 juice, were wrapped in nylon bags for burial at different depths in field plots. Two experiments in the same field area were made at Davis, CA. The soil was solarized from July 5 to August 6. The nylon bags of inoculum were buried in moist soil (70% field capacity) at depths of 15, 30, and 45 cm in six solarized and six nonsolarized plots (12 m sq.). Soil temperatures during the solarized period were monitored at 1-hr intervals throughout the experimental period at the soil depths

where the inocula were buried. Isolate survival was determined by removing and plating soil on selective agar medium from the nylon bags at both 2 and 4 wk after initiating solarization in both field experiments. Soil samples of the individual treatments also were baited with peach leaf disks to determine the survival of the isolates.

## Results

Propagules of *P. cinnamomi* (mainly chlamydospores) and the low-temperature isolates of *P. megasperma* (mainly oospores) were killed in moist soil and infected walnut twigs within 20 min at 45 C (Table 1). In contrast, 30 min at 45 C was needed to kill all propagules of *P. cactorum*, whereas propagules of *P. megasperma* (high-temperature isolate) were still viable after 30 min at 45 C. Propagule survival was a function of time x temperature relationships; with increasing temperature and time, the incidence of peach leaf disks infected by the *Phytophthora* spp. from moist soil was progressively lower. Moreover, thermal inactivation of the isolates of *Phytophthora* spp. in walnut twigs was similar to that in moist soil and was inversely related to the exposure time and temperature (Table 1). Based on the percent recovery from pieces of bark tissue, *P. megasperma* (high-temperature isolate) survived at all temperatures; however, exposure at 45 C for 20 and 30 min resulted in the greatest reduction in recovery compared with untreated infected tissue. In contrast, *P. cinnamomi*, *P. cactorum*, and *P. megasperma* (low-temperature isolate) were not recovered when infected tissue was exposed for 30 min at 45 C. Linear regression analyses of the percent survival of the isolates in walnut tissue were similar to those for survival in soil (data not shown). Slope values ranged from -0.198 to -4.668 and coefficients of determination were significant.

Factorial analyses of variance on the effect of temperature and time on the survival of the *Phytophthora* spp. in moist soil and walnut twigs indicated highly significant interactions ( $P < 0.0001$ ) among species, time, and temperature (Table 2).

### Survival of *Phytophthora* spp. during soil solarization

Survival of the isolates of *Phytophthora* spp. was dependent on the soil temperatures achieved during the solarization period. Soil temperatures were recorded hourly at 15-, 30-, and 45-cm soil depths in both solarized and nonsolarized plots in each field experiment, and daily mean temperature fluctuations are shown in Figure 1. The maximum temperature in the solarized plots was 45 C at 1600 hr at the 15-cm depth, whereas 32 C was reached in nonsolarized soil at the same depth. At 30 cm in solarized soil, the maximum temperature was 37 C, whereas the maximum temperature was less than 30 C in nonsolarized soil.

Among the isolates of *Phytophthora*, *P. cinnamomi* was the most

sensitive to soil solarization in both field experiments; propagules were killed within 2 wk at both the 15- and 30-cm depths (Table 3). *P. cactorum* was fairly sensitive to soil solarization, and all propagules were inactivated within 2 wk at the 15-cm depth at field site 1; however, the results differed in field site 2. In the soil samples retrieved after 2 wk, no colonies were recovered from the leaf disks, whereas colonies developed from 5% of the leaf disks at 4 wk.

The only isolate of *P. megasperma* used in the field tests was the high-temperature isolate; it survived 4 wk of soil solarization at all soil depths (Table 3), with 85 and 98% reductions in colonization of the leaf disks at the 15-cm depth in field sites 1 and 2, respectively.

Factorial analyses of variance on the effect of soil solarization after 2 and 4 wk on the survival of the *Phytophthora* spp. indicated highly significant relationships among time, species, soil depths, and blocks ( $P < 0.0001$ ) for field site 1. Also, for field site 2, the interactions of species, soil depth, and blocks were significant (Table 4). In site 2, time was less a significant factor. Within 2 wk, soil solarization had reduced the survival of the *Phytophthora* spp. as effectively as 4 wk of solarization. Differences among the blocks were highly significant, i.e., in solarized blocks the survival of the *Phytophthora* spp. was 20-40%, whereas in nonsolarized blocks it was 94-96%.

## Discussion

In the present study, the thermal sensitivity of the isolates in infested soil was similar to their heat sensitivity in infected walnut twigs. Temperature X time exposures that reduced the recovery of the isolates more than 95% from walnut bark tissue and from peach leaf disks in flooded soil samples under laboratory conditions, reflected the killing thresholds under field conditions. The linear relationship between death rate of fungal structures as a function of exposure time and temperature has been previously reported. The marked reduction in survival of the isolates of *Phytophthora* in soil samples buried in solarized plots was apparently attributable to the direct effect of elevated soil temperatures on the fungal propagules. These results indicate the potential of postplant soil solarization in orchards for the nonchemical control of soilborne *Phytophthora* species which are among the most injurious to orchard crops.

Table 1. Percent recovery of chlamydospores of *Phytophthora cinnamomi* and oospores of *P. cactorum* and *P. megasperma* (high- and low-temperature isolates) at four temperatures in moist soil and walnut twigs

Phytophthora species	Temperature (C)	Moist soil <sup>a</sup>				Walnut twigs <sup>b</sup>			
		5	10	20	30	5	10	20	30
<i>P. megasperma</i> (high temperature)	30	93 <sup>c</sup>	90	73	67	87	83	77	63
	35	93	87	73	60	83	77	70	47
	40	90	77	53	27	83	73	57	17
	45	83	57	10	6	83	60	13	3
Control		100	100	100	97	100	97	97	97
<i>P. megasperma</i> (low temperature)	30	93	83	63	57	93	70	60	47
	35	83	73	57	47	83	60	50	37
	40	73	67	33	3	73	60	27	3
	45	67	47	0	0	63	37	0	0
Control		100	100	97	97	100	100	97	93
<i>P. cactorum</i>	30	97	87	73	63	93	77	67	63
	35	97	83	70	50	83	73	63	47
	40	93	76	53	23	77	57	47	17
	45	77	53	7	0	73	47	7	0
Control		100	100	97	93	100	100	97	97
<i>P. cinnamomi</i>	30	93	77	67	57	90	83	63	47
	35	93	77	63	47	87	80	60	37
	40	90	57	40	13	87	70	33	4
	45	73	47	0	0	80	60	0	0
Control		100	100	100	97	100	100	97	97

<sup>a</sup> Percentage of peach leaf disks from which colonies developed when plated on PARP selective medium. Before plating, disks were placed in flooded soil samples for 48 hr.

<sup>b</sup> Percentage of infected bark pieces from which colonies of *Phytophthora* spp. developed on agar medium after heat treatments.

<sup>c</sup> Average of three replicates per treatment.

Table 2. Factorial analyses of variance on the effect of temperature  $\times$  time on the percent recovery of isolates of *Phytophthora cinnamomi*, *P. cactorum*, and *P. megasperma* from moist soil and walnut twigs<sup>a</sup>

Source	df	Sum of squares	
		Moist soil	Walnut twigs
Species	3	5,054.17*** <sup>b</sup>	4,318.91***
Time	3	84,508.33***	87,218.91***
Temperature	3	59,283.33***	44,786.42***
Replications	2	232.29*	400.04**
Species $\times$ time	9	679.17*	2,450.08***
Species $\times$ temperature	9	754.17*	45.10
Species $\times$ replications	6	567.71*	523.46*
Time $\times$ temperature	9	12,333.33***	13,435.92***
Time $\times$ replications	6	101.04	360.96
Temperature $\times$ replications	6	376.04	508.46*
Species $\times$ time $\times$ temperature	27	1,112.50	1,507.75
Error	108		

<sup>a</sup> Analysis of variance was done on data from Table 1 using the type III sums of squares method of the general linear models procedure of SAS.

<sup>b</sup> \* = Effects significant at  $P \leq 0.05$ , \*\* = significant at  $P < 0.01$ , and \*\*\* = significant at  $P < 0.001$ .



Table 3. Effect of soil solarization (using three soil depths and two time periods) on percent recovery of *Phytophthora cinnamomi*, *P. cactorum*, and *P. megasperma* (high-temperature isolate)

	Depth	<i>P. cinnamomi</i>		<i>P. cactorum</i>		<i>P. megasperma</i>	
Treatment	(cm)	2 wk	4 wk	2wk	4 wk	2 wk	4wk
Field site 1							
Solarized <sup>a</sup>	15	0.0 <sup>b</sup>	0.0	0.0	0.0	26.7	1.7
	30	0.0	0.0	10.0	3.3	76.7	40.0
	45	38.3	0.0	55.0	13.3	86.7	56.7
Nonsolarized	15	96.7	88.3	96.7	90.0	100.0	93.3
	30	95.0	91.7	100.0	93.3	98.3	95.0
	45	100.0	93.3	100.0	95.0	100.0	98.3
Control <sup>c</sup>		95.0	96.7	96.7	98.3	100.0	98.7
Field site 2							
Solarized	15	0.0	0.0	0.0	5.0	23.3	15.0
	30	0.0	0.0	8.3	15.0	63.3	48.3
	45	20.0	3.3	21.7	26.7	91.7	53.3
Nonsolarized	15	93.3	90.0	100.0	95.0	100.0	98.3
	30	93.3	93.3	95.0	95.0	93.3	98.3
	45	90.0	95.0	91.7	100.0	96.7	100.0
Control <sup>c</sup>		100.0	96.7	98.3	100.0	98.3	95.0

<sup>a</sup> Transparent polyethylene tarps 25- $\mu$ m thick were placed on the soil on 7 July and removed 6 August 1989 at Davis, CA.

<sup>b</sup> Percentage of peach leaf disks from which colonies developed when plated on PARP selective medium. Before plating, disks were placed in flooded soil samples for 48 hr. Average of six replicates per treatment.

<sup>c</sup> The controls were maintained in laboratory at 22-24 C.

Table 4. Factorial analyses of variance on the effect of soil solarization on recovery after 2 and 4 wk of isolates of *Phytophthora cinnamomi*, *P. cactorum*, and *P. megasperma* at three soil depths<sup>a</sup>

Source	df	Sum of squares	
		Site 1	Site 2
Time	1	8,689.35****	6,446.30
Species	2	21,823.15***	58,125.00***
Depth	2	15,270.37***	26,944.44**
Block	5	289,057.87***	243,750.00***
Replications	1	104.17	3,266.67
Time $\times$ species $\times$ depth $\times$ block	20	1,370.37	81,903.70
Time $\times$ species	2	650.93**	14,200.93
Time $\times$ depth	2	1,837.04***	9,848.15
Time $\times$ block	5	3,157.87***	22,970.37
Time $\times$ species $\times$ depth	4	1,201.85***	19,862.96
Time $\times$ species $\times$ block	10	2,210.19***	47,999.07
Time $\times$ depth $\times$ block	10	3,007.41***	39,918.52
Species $\times$ depth	4	3,696.30***	25,855.56
Species $\times$ block	10	15,837.96***	73,975.00*
Species $\times$ depth $\times$ block	20	4,875.93***	88,644.44
Depth $\times$ block	10	11,507.41***	56,688.89
Error	107		

<sup>a</sup> Analysis of variance was done on data in Table 3 using the type III sums of squares method of the general linear model procedure of SAS.

\* = Effects significant at  $P \leq 0.05$ , \*\* = significant at  $P < 0.01$ , and \*\*\* = significant at  $P < 0.001$ .

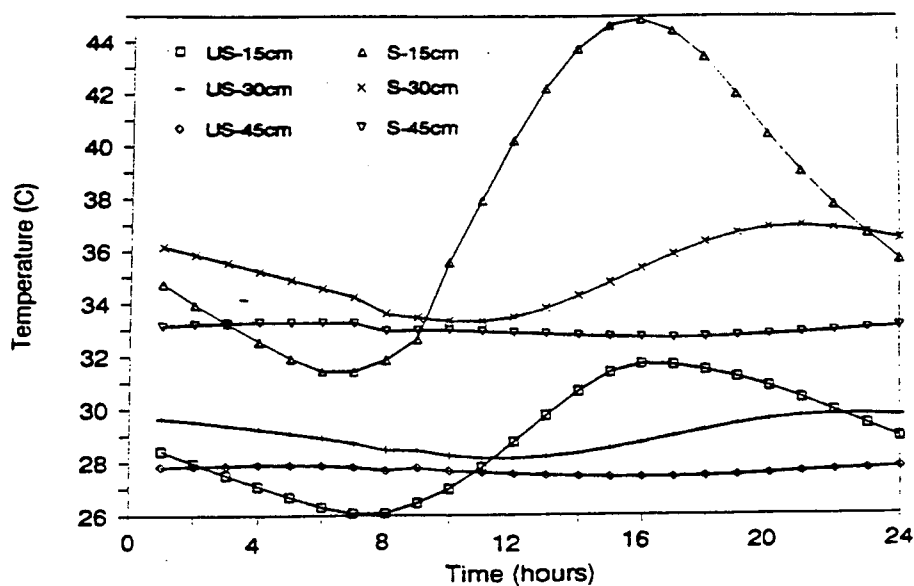


Fig. 1. Daily mean temperature fluctuations (5 July to 6 August 1989) in solarized (S) and unsolarized (US) field sites at depths of 15, 30, and 45 cm. Each value is an average of two measurements in two field sites.

1 Establishment of Apricot and Almond Trees Using Soil Mulching  
2 with Clear and Black Polyethylene Film: Effects on Verticillium  
3 Wilt and Tree Health.

4 J. J. STAPLETON

5 Statewide IPM Project, Kearney Agricultural Center, University of  
6 California, Parlier, CA 93648, USA

8 R. J. WAKEMAN and J. E. DeVAY

9 Department of Plant Pathology, University of California, Davis,  
10 CA 95616, USA

11 First-leaf apricot (Prunus armeniaca) and almond (P. dulcis)  
12 trees were planted in soil infested with Verticillium dahliae and  
13 mulched with clear or black polyethylene film, or not mulched, in  
14 the San Joaquin Valley of California, March-August 1990. During  
15 the 19 wk mulching treatment, summer soil temperatures reached as  
16 high as 46, 41, and 33°C at 18 cm depth; and 41, 37, and 32°C at  
17 30 cm depth under clear film, black film, and no film, respec-  
18 tively. Trees mulched from the time of planting with clear  
19 polyethylene (solarization) did not survive or grow as well as  
20 those mulched with black film or not mulched. Incidence of  
21 foliar symptoms due to Verticillium wilt was reduced 86-100% in  
22 both apricot and almond trees by black, as well as clear film  
23 mulch the following season. Incidence of vascular discoloration  
24 symptoms of trunks and primary scaffolds due to Verticillium wilt  
25 was similarly reduced by both mulches. Mulching with black  
26 polyethylene film gave better overall results than solarization  
27 with clear film, since the intermediate soil temperatures pro-

duced did not chronically harm trees as judged by tree survival and annual growth of trunk diameter, yet the prolonged period of soil heating provided control of *Verticillium* wilt equivalent to that of solarization. These studies also gave further evidence that in-season mulching can be used to conserve water during establishment of new orchards or replant trees in warm, arid climates.

#### INTRODUCTION

*Verticillium* wilt, caused by the fungus *Verticillium dahliae*, is a vascular wilt disease which commonly attacks young *Prunus* trees, including several species which are important fruit and nut crops. Symptoms include wilting, defoliation, and death of scaffolds, shoots, and sometimes entire trees. Colonization and plugging of xylem vessels produces vascular discoloration of infected tissue (Parker, 1959).

In the San Joaquin Valley of California, *Prunus* orchards are frequently established on ground in which susceptible crops or weeds previously grew, and traditionally, chemical pesticides have not been consistently effective in disinfesting soil of *V. dahliae* (authors, unpublished). Under extreme conditions, more than 60 propagules of *V. dahliae*/g soil are recovered, while less than 5/g are sufficient to cause a high incidence of wilt in susceptible *Prunus* spp. (authors, unpublished). *Verticillium* wilt is a disease of young *Prunus* trees, usually second- through sixth-leaf, and usually kills shoots and scaffolds, rather than entire trees (Taylor & Flentje, 1968; Ciccicarese et al, 1990).

## Thermal Sensitivity of Three Species of *Phytophthora* and the Effect of Soil Solarization on Their Survival

C. JUAREZ-PALACIOS, R. FELIX-GASTELUM, R. J. WAKEMAN, E. J. PAPLOMATAS, and J. E. DeVAY, Department of Plant Pathology, University of California, Davis 95616

### ABSTRACT

Juarez-Palacios, C., Felix-Gastelum, R., Wakeman, R. J., Paplomatas, E. J., and DeVay, J. E. 1991. Thermal sensitivity of three species of *Phytophthora* and the effect of soil solarization on their survival. *Plant Dis.* 75:1160-1164.

Heat sensitivity and the effect of soil solarization on the survival of hyphae and spores of *Phytophthora cinnamomi*, *P. cactorum*, and *P. megasperma* were determined in inoculated walnut twigs and artificially infested Reiff silty clay loam soil. Viable chlamydospores of *P. cinnamomi* were not detected in infested soil exposed to 45 C for 20 min. In contrast, oospores of a high-temperature isolate of *P. megasperma* survived exposure for 30 min at 45 C, whereas *P. cactorum* was killed within 30 min at 45 C. In field studies, solarized soil reached a maximum temperature of 45 C at the 15-cm depth and 33 C at the 45-cm depth, compared with nonsolarized soil where maximum temperatures of 31 and 28 C were recorded at 15 and 45 cm, respectively. No activity of *P. cinnamomi* was detected in infested soil after 2 wk at the 30-cm depth or after 4 wk at the 45-cm depth in solarized soil, whereas there was little or no reduction in survival in nonsolarized soil. *P. cactorum* withstood the effects of soil solarization at the 30- and 45-cm depths but was killed within 2 wk at the 15-cm depth, with little or no change in the percentages of infection of peach leaf disks used as bait in nonsolarized soil. Some propagules of the high-temperature isolate of *P. megasperma* survived the treatment, although the percentage of leaf disk infection was greatly reduced after soil solarization at the 15-cm depth during 4 wk. The heat sensitivity of the isolates of *Phytophthora* in laboratory experiments closely reflected their inactivation in solarized soil and indicated the possible use of soil solarization for management of these pathogens.

Species of *Phytophthora* are among the most important plant pathogens in different crops under different cropping systems (9-11,14). *Phytophthora cinnamomi* Rands, *P. cactorum* (Lebert & Cohn) J. Schröt., and *P. megasperma* Drechs. cause major losses in many herbaceous and perennial crops and agricultural regions worldwide (1,2,11,25,26). Root and crown rot diseases of fruit trees often involve a complex of several pathogenic *Phytophthora* spp. in a single host (11). Soilborne species of *Phytophthora* can be disseminated in soil on cultivation equipment, in planting stock (9,11,26), and in runoff and irrigation water (11,13). Once established in soil, their eradication is difficult (11).

Several approaches are used to minimize losses caused by *Phytophthora* root and crown rot in perennial crops, including soil-water management (10,12) and planting trees in raised beds (19). Preplant soil fumigation has also been used, but its effectiveness is limited by reinfestation of soil after the treatment

(12). Another approach involves soil solarization, either for preplant or post-plant management of soilborne diseases. It has the added advantages of being much less expensive than soil fumigation and causes an increased growth response and yield of crop plants (4,7,8,17,21-23) and the control of most weed species (23). However, water management is the main approach for cultural control of *Phytophthora* spp., although soil fumigation and soil solarization have also been effective (1,16). Among the *Phytophthora* spp. that have been controlled by soil solarization are *P. cambivora* (Petri) Buisman in cherry (24), *P. cryptogea* Pethybr. & Lafferty in gerbera (5), and *P. cinnamomi* in avocado in naturally infested soils (16).

The objectives of our study were to compare the thermal sensitivity of *P. cinnamomi*, *P. cactorum*, and *P. megasperma* in inoculated walnut twigs and in artificially infested soil and to determine the effect of soil solarization on the survival of these species in infested soil at different depths.

### MATERIALS AND METHODS

**Isolates of *Phytophthora* species.** Isolates of *Phytophthora* spp. were provided by S. M. Mircetich (University of California, Davis). They consisted of a peach isolate (2-1-4) of *P. cinnamomi*, an isolate (APP 204-C) of *P. cactorum* from apple, and two isolates (22-2-3 and 22-2-5) of *P. megasperma* from peach. All were pathogenic on English walnut

(9). Isolates 22-2-3 and 22-2-5 correspond to types having low optimal temperatures and large oogonia and high optimal temperatures and small oogonia, respectively, previously described by Wilcox and Mircetich (25). The optimal temperature for growth of the low-temperature group of *P. megasperma* is 24 C, with poor growth at 30 C and no growth at 33 C. In contrast, the optimal temperature for growth of the high-temperature group is 30 C, with vigorous growth at 33 C and poor growth at 36 C. All isolates of *Phytophthora* spp. used were maintained on PARP selective agar medium (6) in darkness at 20 C. The method of Mircetich et al. (15) was used for the preparation of chlamydospores from *P. cinnamomi*, whereas oospore production by *P. cactorum* and *P. megasperma* was induced by the method of Snch (20).

**Thermal sensitivity of the *Phytophthora* isolates under controlled conditions.** Oospores and chlamydospores were mixed with moist soil (Reiff silty clay loam, approximately 70% field capacity) to give approximately 600 cfu/g of soil. Infested soil (25 cm<sup>3</sup>) was placed in polyethylene bags, which then were partially evacuated to reduce air insulation, and the soil was spread in the bags to make a thin layer (1-2 mm) deep. The bags of soil then were immersed in water at 30, 35, 40, or 45 C for 5, 10, 20, or 30 min. After treatment, the infested soil was placed in petri dishes and flooded with tap water. Survival of propagules was determined by placing 10 leaf disks (6 mm diameter) from young Lovell peach leaves (*Prunus persica* (L.) Batsch) on the flooded soil at 18 C for 48 hr; disks then were plated onto PARP and incubated at 21 C. Each treatment was replicated three times. Percent incidence of infected leaf disks (disks from which *Phytophthora* colonies developed) was used to determine survival of the *Phytophthora* spp.

The laboratory experiment on thermal sensitivity of the *Phytophthora* spp. (all pathogens of English walnut) was repeated using inoculated walnut twigs according to the method of Matheron and Mircetich (9). The isolates were grown on V8 juice agar (10) for 4 days at 21 C, then agar disks (6 mm diameter) were cut from the edges of the colonies for inoculation of freshly cut walnut twigs. The English walnut twigs, approximately 1 cm in diameter and 20 cm long, were inoculated by placing an agar disk with

Present address of first author: Campo Agrícola Experimental, Km. 1 Carret. Tecamachalco-tochtepec, Apartado Postal No. 43, C.P. 75480, Tecamachalco, Pue., Mexico. Present address second author: Sinalopasta, S. A. de C.V. Apartado Postal No. 185, Guasave, Sinaloa, Mexico.

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USE OF POLYMER MULCHES IN INTEGRATED PEST MANAGEMENT  
PROGRAMS FOR ESTABLISHMENT OF PERENNIAL FRUIT CROPS

J. J. Stapleton  
Statewide UC/IPM Project  
University of California  
Cooperative Extension  
733 County Center III  
Modesto, California 95355  
U.S.A.

W. K. Asai  
University of California  
Cooperative Extension  
733 County Center III  
Modesto, California 95355  
U.S.A.

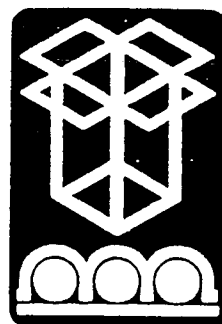
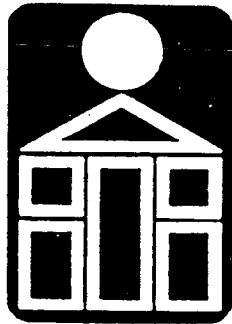
J. E. DeVay  
Department of Plant Pathology  
University of California  
Davis, California 95616  
U.S.A.

Abstract

Field experiments were done to determine the benefits and drawbacks of using polyethylene film mulches, primarily soil solarization, for pest control in establishment and replanting of perennial fruit crops. Experimental crops included apple and pecan trees, and grapevines planted in the semi-arid San Joaquin Valley of California. Soil temperatures at 15-23 cm depth usually were raised by 10-18 C under transparent (solarization), and 8-12 C under black film mulching. Both treatments reduced natural population densities of Pythium ultimum and Verticillium dahliae 55-97% in the 0-23 cm soil depth range. Viability of artificially-infested sclerotia of Sclerotium rolfsii in contact with field soil at 2.5 cm soil depth was reduced by more than 95% after 30 days of mulching by either film treatment. Mulches were less effective in reducing viability of S. rolfsii at 10 or 30 cm depth, especially when sclerotia were incubated without soil contact. Solarization did not noticeably affect survival of grape phylloxera (Daktulosphaira vitifoliae) on grapevine roots. Percent ground cover by winter or summer weeds was reduced more than 82% after solarization or black film mulching. No evidence of tree or vine damage was observed after mulching. Pre-irrigated pecan trees were grown for an entire season without further irrigation when mulched in May with either transparent or black film. Although no significant crop growth responses were found, a consistent trend of increased crop vegetative growth or fruit yield was observed after film mulching.

1. Introduction

There is a decreasing number of registered soil fumigation and disinfestation pesticides available in California, mainly due to the risk of polluting groundwater or soil. In addition, significant consumer demand exists for food produced without pesticides or pesticide residues, and for sustainable agricultural production



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STAPLETON

USE OF IN-SEASON POLYETHYLENE MULCHING FOR ESTABLISHMENT OF  
DECIDUOUS FRUIT AND NUT TREES IN THE SAN JOAQUIN VALLEY: EFFECTS ON  
PATHOGEN NUMBERS AND TREE SURVIVAL.

James J. Stapleton  
Statewide Integrated Pest Management Project  
Kearney Agricultural Center  
University of California  
9240 S. Riverbend Ave.  
Parlier, CA 93648, U.S.A.

R. J. Wakeman and J. E. DeVay<sup>1</sup>  
Department of Plant Pathology  
University of California  
Davis, CA 95616, U.S.A.

Abstract: Field experiments conducted during the past several years in the northern San Joaquin Valley have shown that many species of deciduous fruit and nut trees can tolerate in-season polyethylene mulching with minimal damage to trees. During the mulching period, soilborne populations of undesirable fungi, nematodes, and weeds were usually greatly reduced through the process of soil solarization, and soil moisture was conserved. In 1990, replicated, bareroot transplants of apricot and almond trees were mulched with either transparent or black, 4-mil polyethylene film, or not mulched, near Fresno in the hotter, central San Joaquin Valley. The soil was infested with the vascular wilt pathogen, Verticillium dahliae. Mulched trees were not irrigated between planting in March and mid-August, while control trees were irrigated on a ca. bi-weekly schedule. At the end of the 1990 growing season, numbers of fungal propagules were significantly reduced by both mulch treatments in soil near almond trees, but only by transparent mulch near apricot. In-season mortality of trees mulched with polyethylene film was statistically no greater than the control, except for almond trees mulched with transparent film which did not tolerate the treatment.

Introduction

In 1982, soil solarization using clear, polyethylene film mulch was reported to have controlled Verticillium wilt, caused by the soilborne, phytopathogenic fungus Verticillium dahliae,

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**SUBJECT:** Production and culture

**TITLE:** Solarization effective as a soil disinfestation technique for strawberry production

**AUTHOR:** T.K. Hartz, J.E. DeVay<sup>1</sup> and C.L. Elmore<sup>1</sup>  
Department of Vegetable Crops  
University of California  
Davis, CA 95616

**ADDITIONAL INDEX WORDS:** *Fragaria x ananassa* Duch., *Verticillium dahliae*, *Phytophthora* spp., fumigation, weed control, polyethylene mulch.

**ABSTRACT:**

Soil solarization, alone and in combination with metam sodium, was evaluated as an alternative to methyl bromide/chloropicrin (MBC) fumigation, the standard soil disinfestation technique in the California strawberry industry. Tests were conducted in two consecutive annual production cycles in Irvine, California, an environment representative of the coastal strawberry production area. Solarization treatments were applied from late July through September for October plantings. All treatments were equally effective in reducing baited populations of *Phytophthora cactorum* (1989-90) and *P. citricola* (1990-91) when compared to pathogen survival in untreated soil. Both solarization and MBC were highly effective in reducing inoculum of *Verticillium dahliae* in 1989-90, but MBC gave superior control in 1990-91. Solarization gave significant control of annual weeds but was less effective than MBC in that regard. In 1989-90, solarization alone increased strawberry yield 12% over untreated plots; when combined with metam sodium (MS) yield increase was 29%, equivalent to that achieved with MBC fumigation. All treatments were equally effective in increasing yields in the 1990-91 test.

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<sup>1</sup> Department of Plant Pathology and Department of Botany, respectively. The support of the California Strawberry Advisory Board and the Kay Mukai Research Foundation is gratefully acknowledged.

PHYTOPATHOLOGY (IN PRESS) 1992

VERTICILLIUM WILT OF PRUNUS ORCHARD TREES CONTROLLED WITH POLYETHYLENE MULCH. J. J. Stapleton, E. J. Paplomatas, R. J. Wakeman, and J. E. DeVay, University of California, Kearney Agricultural Center, Parlier 93648.

First-leaf apricot (Prunus armeniaca) and almond (P. dulcis) trees were planted in soil infested with Verticillium dahliae, and either mulched (solarized) with clear or black polyethylene film, or not mulched, during March-August 1990. Soil temperatures were elevated in mulched treatments. Trees mulched with clear polyethylene did not survive or grow as well as those mulched with black film. Foliar symptoms in experimental trees were reduced 86-100% by film mulches. Vascular discoloration in trunks and scaffolds were similarly reduced by mulches. Black film gave better results than the clear, since intermediate soil temperatures controlled V. dahliae but did not chronically injure trees. Mulched trees were not irrigated between March and August.

#### Description of cooperation

The principal investigators have been in close cooperation for many years, and especially in the framework of various BARD projects. The highlight of this cooperation, is the co-editing by J. Katan and J.E. DeVay of a book on "Soil Solarization" (CRC Press, 1991, Boca Raton), which contains 18 chapters written by 32 authors. The joint and parallel studies carried out in Israel and the USA (in the framework of three BARD projects) and the continuous exchange of ideas, during many years, stimulated the writing of this book. Frequent exchanges of ideas and information between Professors DeVay and Katan and visitations to Davis, CA, by Drs. Y. Pinkas, Cohen and A. Gamliel and meetings between Drs. DeVay Katan and Stapleton in various occasions in the USA and Europe, have been invaluable to the success and highly significant progress of research on soil solarization. The BARD project stimulated cooperation and positive interaction between scientists in both the U.S. and in Israel in achieving the research goals set forth in the BARD proposal.

The special supplementation of grant support and extension of the grant period for this project from BARD enabled Dr. Hisham Yunis to spend 1 year working with Dr. DeVay and primarily with Dr. James J. Stapleton at the Kearney Agricultural Center, University of California, at Parlier, CA. Moreover, the simultaneous presence of Dr. Gamliel (the former student of Dr. Katan) on a postdoctoral appointment with Dr. Stapleton, allowed significant cooperation and progress in the research of these Israeli visitors.

The following statement summarizes the activities of Dr. Hisham Yunis:

Dr. Yunis was involved in a number of research and extension projects including thermal inactivation studies on Pythium ultimum, disease management in grapes, and assisted with solarization studies. Dr. Yunis also went on tours with various Extension personnel to study agricultural production methods in California. He also gave several seminars during his work at the Kearney Agricultural Center. The main focus of Dr. Yunis' research was an in vitro study of effects of soil heating on native population of P. ultimum. He used three representative temperature regimes commonly found during solarization in the San Joaquin Valley, both continuous temperature and diurnal heat flux, to obtain LD<sub>50</sub> values. In a noncontinuous heating regime, at the lower 42 C temperature, several days were required to inactivate the

fungal propagules. At 45 C the time necessary to reach LD<sub>50</sub> was reduced to hours, and at 48 C, only minutes were required to inactivate the fungal propagules. Dr. Yunis plans to publish an article on this research in a scientific journal.

Evaluation of the research achievements with respect to the aims of the original research proposal

Although solarization has been studied and used since 1976, the emphasis was put mainly on annual crops, as preplant soil disinfestation. This project addresses the difficult task of solarizing a soil in which plants are growing (postplant solarization). The main conclusion is, that many trees, although suffering during heating by solarization, they survive it, surprisingly. Moreover, in some cases their growth even improves at later stages. To our best knowledge, our studies show for the first time physiological changes occurring in the plant during solarization. Not less important, is the finding that postplant solarization conserves water and that black polyethylene for postplant solarization has special advantages over the conventional transparent polyethylene. In the framework of this project, many pathogens and trees were studied. Hopefully, this will encourage other workers to extend our work, or adapt it for other conditions.

Of great significance and progress in the usefulness of soil solarization is its effectiveness as a postplant nonchemical treatment of soil to manage soilborne pathogens and pests. This project has pioneered some of the aspects of the use of postplant solarization and characterized the different parameters affecting its effectiveness. The experiments in the various studies have provided valuable insights into the use of solarization and have met the goals of the BARD project. With increasing constraints on the uses of chemical pesticides, the importance of soil solarization cannot be underestimated as a major contribution in the nonchemical control of plant diseases and pests. The support of BARD for projects on soil solarization have been of major importance in the development of this alternative to chemical pesticides.

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