Recovery of Olive Trees with Verticillium Wilt After Individual Application of Soil Solarization in Established Olive Orchards

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ABSTRACT

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Evidence was obtained suggesting that olive trees with Verticillium wilt (caused by *Verticillium dahliae*) could be recovered after soil solarization of individual 10- to 15-yr-old trees. Rate of recovery of trees in solarized soil significantly exceeded natural recovery of untreated control trees and was attributed to the lack of root reinfection. Microsclerotia of *V. dahliae* were nearly eliminated in the soil around treated trees, whereas propagules of *Talaromyces flavus*, a known antagonist of *V. dahliae*, not only survived solarization but also increased in treated soil, compared with untreated control soils. Because the differences in symptom remission or recovery persisted for at least three successive years, soil solarization of individual diseased trees, when combined with chemical weed control, could be of practical value in controlling Verticillium wilt of olive trees.

Verticillium wilt (caused by *Verticillium dahliae* Kleb.) of olive (*Olea europapea* L.), pistachio nut (*Pistacia vera* L.), and prunus (Rosaceae) trees constitutes one of the most difficult problems in tree pathology. The impact of the disease is particularly severe in intercropped orchards during the first years after the establishment of the groves but tends to become rather mild with aging.

Trees with Verticillium wilt in young prunus orchards, especially almond and peach, tend to overcome the disease after an acute phase and escape new infections (25). Pistachio nut trees suffer less several years after establishment. Olive trees, however, possess superficial root systems and are almost always vulnerable to the pathogen. This is particularly true in irrigated groves of susceptible olive cultivars, such as the extremely susceptible Greek cultivar Konservolia. Transient symptom development of Verticillium wilt of apricot and olive (16,27) is possibly attributable to the inactivation of the pathogen in the annual ring. Diseased trees are also able to recover and escape the disease after pruning, provided that future reinfestation through the roots can be avoided. Consequently, any factor(s) able to reduce the inoculum level of *V. dahliae* in the soil could contribute to the recovery of diseased trees.

Verticillium wilt of olives is a limiting factor for the cultivation of high-yielding, excellent quality cultivars in Greece (17), the Mediterranean basin (4,5,23), and California (6,26). Currently available fungicides (mainly carbendazim) are unable to successfully control the pathogen (12). Furthermore, resistant olive rootstocks are acceptable in California (6), but their behavior is questionable in other countries (20). Because no other measures could effectively prevent symptom development in established groves, experiments to determine the effect of soil solarization on individual trees were carried out between 1980 and 1983 in olive orchards in Greece (19). The soil around the diseased trees was covered with transparent polyethylene sheets (6 × 6 m) and reduction in symptom expression apparently demonstrated recovery of the treated trees compared with the untreated controls. No visual damage was noticed on the roots of treated trees. Similarly, in California (1) *V. dahliae* could be controlled in established pistachio nut trees with continuous soil solarization. High soil temperatures in mulched soil did not exhibit any detrimental effect on the root system.

The present work was a large-scale experiment designed to evaluate the effectiveness of soil solarization of individual trees in controlling Verticillium wilt of olive trees over a 3-yr period. The study involved analysis of the effect of a single treatment on the natural population of *V. dahliae*, estimation of symptom remission or recovery of olive trees, and detection and quantification of fungal antagonists to *V. dahliae* in soil. Finally, it focused on the longevity of the effect and attempts to elucidate the involvement of heat-tolerant antagonists of *V. dahliae* in the rhizosphere in the effectiveness of the method.

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MATERIALS AND METHODS

Experimental site and disease assessment. The experiment was conducted in Magnesia County, Greece, in a field with a sandy loam soil and more than 700 10- to 15-yr-old olive trees (cv. Konservolia). The diameter of the tree canopy ranged between 3 and 5 m. At the initiation of the experiment in July 1984, more than 200 trees exhibited symptoms typical of Verticillium wilt. Foliar symptoms, including dull green, internally rolled, or necrotic leaves and defoliated twigs, were evaluated on a scale of 0–4 based on the percentage of the affected foliage, where 0 = tree healthy; 1 = up to 25% (mild symptoms); 2 = up to 50% (intermediate symptoms); 3 = up to 75% (severe symptoms); and 4 = more than 80% diseased foliage (tree nearly dead to dead).

The effectiveness of soil solarization of individual trees in controlling Verticillium wilt or in contributing to symptom remission was evaluated by recording the disease indices of each group of trees in July 1985, January 1986, July 1986, and January 1987.

Application of soil solarization. Soil solarization was initiated on 16–18 July 1984 in 57 diseased olive trees with symptom ratings of from 1 to 3 with a mean initial rating of 1.6 (30 trees graded 1, 17 graded 2, and 10 graded 3). Another group of 118 diseased olive trees with symptom ratings from 1 to 3 and with a similar initial mean of 1.6 (58 trees graded 1, 44 trees graded 2, and 16 trees graded 3) were used as untreated controls. Before the solarization, weeds were destroyed mechanically and the upper 5 cm of the soil of each tree was removed to the periphery of the projection of the tree canopy. This soil was used both for allowing uniform irrigation and tightly anchoring the polyethylene sheet (100 μm thick) over an area covering 6 × 6 m of soil. Topsoil was scraped off control trees for even tree irrigation. Trees were irrigated to the soil saturation level (300–350 L of water per tree) and plastic tarps were placed on the soil. Solarization lasted for at least 2 mo, after which the sheets began to disintegrate and split apart. Maximum and minimum values of soil temperatures were recorded continuously with a three-point distal thermograph at depths of 10 and 20 cm in solarized and at 20 cm in unsolarized soil.

Treatments after solarization. To avoid dissemination of microsclerotia of *V. dahliae* from untreated to mulched soil, no rotovation was practiced after solarization. Therefore, weed control was based on the use of herbicides. Applications of herbicides were made in March 1985 (a commercial mixture of aminotriazole, diuron, linuron, and monolinuron), May 1985 (glyphosate), July 1985 (paraquat), and October 1985 (glyphosate). Glyphosate and paraquat were used periodically in 1986 and 1987. To facilitate assessment of symptom remission or tree recovery, diseased branches were removed immediately after the initial determination of the disease index within each group of treated or untreated control trees.

Assessment of population of microsclerotia of *V. dahliae*. Natural populations of microsclerotia of *V. dahliae* were determined from soil samples taken at soil depths of 5–20 cm. Soil samples were collected arbitrarily from 10 treated and 10 untreated olive trees. Each sample consisted of four 100- to 200-g subsamples taken in a diagonal pattern from four sites around the sampled tree and mixed to form a single sample. Sampling and estimation of density of microsclerotia were carried out in January 1985, 4 mo after the solarization. Second and third soil assays were carried out after 1 and 2 yr, respectively. Triplicate 15-g samples of air-dried soil were treated by a wet-sieving procedure (7) with the following modifications: the soil was washed through sieves with 125- and 36-μm openings, and the residue retained on the 36-μm sieve was washed for 30 sec with 40 g/L of NaOCl, transferred to 50-ml tubes, and made up to 30 ml with sterile distilled water. The soil particles were left to precipitate for 30 min and excess water was removed by aspiration. The final 10 ml of residue was spread uniformly over a semiselective growth medium (3) in each of 10 petri dishes and incubated for 21 days at 18 C. Colonies of *V. dahliae* were counted after soil residues were removed from the medium by a gentle stream of tap water.

Isolation and quantification of populations of *Talaromyces flavus* and *Aspergillus terreus*. A selective medium (10) was used to isolate and quantify the natural population of *Talaromyces flavus* (Klöcker) A. C. Stolk & R. A. Samson in the soil rhizosphere (up to 5 mm around the actual root) and rhizoplane in both treated and untreated olive trees. This same medium was also used for the determination of population density of *Aspergillus terreus* Thom in Thom & Church, an antagonist against *V. dahliae* (14,22). Botran 75% at 1.5 mg/L was added to the medium to inhibit growth of *Rhizopus* spp. Three replicate soil samples of 1 g each were suspended in 100 ml of deionized sterile water; 1-ml aliquots were removed during agitation and spread on the medium (three petri dishes per sample). Plates were incubated at 30 C in the dark. Colonies of *T. flavus* or *A. terreus* were counted after 8–10 days of incubation. Soil sampling and analysis were carried out in April and October 1985, January 1986, May 1986, and May 1987 or as otherwise stated in the results.

Occurrence of the pathogen in olive twigs. To confirm the validity of symptom assessment, attempts were made to isolate *V. dahliae* from ostensibly healthy and diseased olive twigs. In 10 different orchards, 22 randomly selected trees with both healthy and diseased branches were assayed for the presence of the fungus in spring 1989. Three twigs with and three without symptoms were collected from each tree, and nine isolations were attempted from each twig.

Assessment of mycorrhizal roots. The effect of solarization on the proportion of mycorrhizal roots was determined by analyzing olive root samples taken from solarized and untreated soil around the trees. Samples collected from soil depths of 5–15 and 15–30 cm were analyzed by V. Pearson-Gianninazzi of INRA at Dijon, France.

RESULTS

Effect of solarization on soil temperatures. The maximum and minimum values of soil temperatures recorded in the unshaded parts around treated trees were usually 9–12 C higher than those of the uncovered soil in the control trees (Fig. 1). Maximum soil temperatures in covered soil reached 58 and 48 C at depths of 10 and 20 cm, respectively, compared with 36 C in the uncovered soil at 20 cm. The minimum soil temperatures were also higher in treated than in untreated soil. The magnitude of the value recorded between 1 and 14 August 1984 is comparable to values reported previously for southeastern Greece (22).

Effect of soil solarization on the percentage of the mycorrhizal roots. Percentage of mycorrhizal roots was greater in the untreated control (85% at 5–15 cm or 64% at 15–30 cm soil depths) than in the solarized soil (45% at 5–15 cm or 25% at 15–30 cm soil depths). However, in the field, the growth rate of the solarized trees was not adversely affected and trees did not develop any abnormal symptoms.

Pathogen isolation. Although 36% of healthy looking twigs were colonized by *V. dahliae*, on the average, fewer than one in nine segments per branch assayed positively for the fungus. Of the diseased twigs, 86% harbored the fungus, with up to 19% positive isolations (two in nine segments) per tree (Fig. 2).

Effect of soil solarization on the populations of microsclerotia of *V. dahliae*. Natural populations of microsclerotia were almost eradicated after soil solarization (Fig. 3A). The values of viable microsclerotia recorded 1 and 2

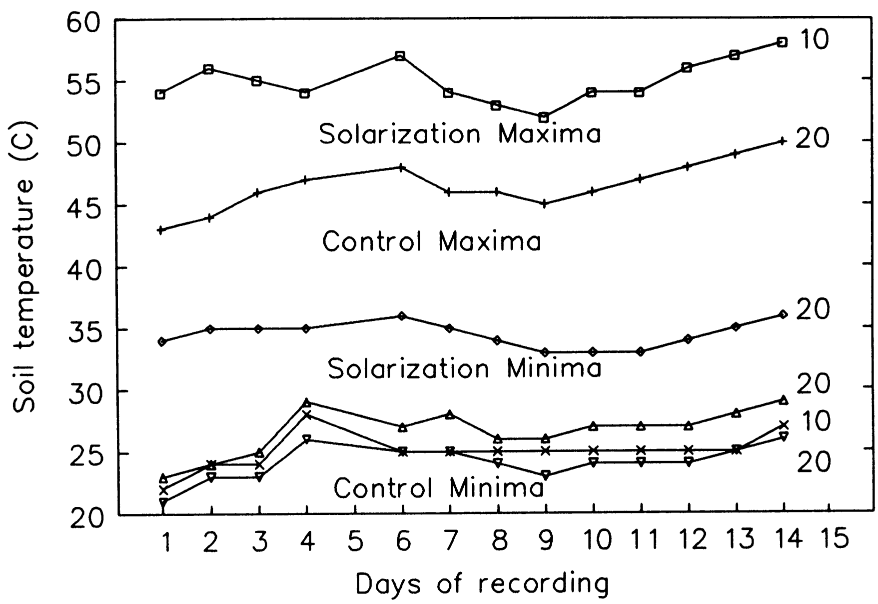

Fig. 1. Maximum and minimum soil temperatures recorded on 14 successive days between 1 and 14 August 1984 in Pteleos region of Magnesia County in east central Greece.

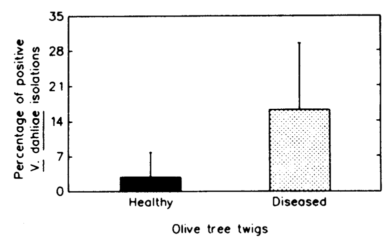

Fig. 2. Occurrence of *Verticillium dahliae* in olive twigs with or without symptoms of Verticillium wilt expressed as percentage of isolations of *V. dahliae*. Three healthy and three diseased twigs were collected from every tree, and nine isolations were attempted for each tree. Olive trees were sampled in February 1989. Treatment effect was significant at $P = 0.0001$.

yr later in the same trees had increased slightly but in general remained significantly lower in treated than in untreated trees. The actual mean figures ± SE are 9 ± 7, 28 ± 11, and 51 ± 20 for solarized and 45 ± 11, 89 ± 11, and 67 ± 24 microsclerotia for untreated soil per 100 g of soil in January 1985, 1986, and 1987 respectively.

Effect of soil solarization on survival and fluctuation of populations of *T. flavus*. In tests in April 1985, *T. flavus*, an antagonist of *V. dahliae*, was present in the soil of the experimental olive grove. Population determinations carried out in rhizosphere soil of the treated or untreated control trees showed a sharp difference in favor of the solarized trees. Further estimations conducted in October 1985, January 1986, May 1986, and May 1987 clearly demonstrated the positive effect of soil solarization on the constant occurrence of the antagonist in solarized soil. Solarization had a pronounced, long-lasting effect on the survival, increase, and maintenance of the antagonistic population in the rhizosphere of 10 treated trees (Fig. 3B). It is also apparent that values for propagule density of *T. flavus* in soil around 10 untreated trees fell drastically in the following years, whereas this drop is observed in both cases in January 1986. An analysis of variance performed on data from a separate individual estimation of *T. flavus* populations carried out in 30 solarized and 30 untreated control trees in January 1986, May 1986, and May 1987 (Table 1) indicated that solarization significantly affected survival and maintenance of populations of *T. flavus* for 2–3 yr after solarization. There was no significant difference, however, among healthy and diseased trees within the same treatment.

Effect of soil solarization on populations of *A. terreus*. Because colonies of *A. terreus* were frequently found in petri dishes containing the selective medium used to estimate populations of *T. flavus*, counts of propagules of *A. terreus* were also made in May 1986 and 1987. This potential antagonist of *V. dahliae* survived solarization and was detected in exceptionally high numbers in both treated and untreated trees. Propagule counts of *A. terreus* were significantly higher in soil from solarized trees with already higher initial populations of *T. flavus* (Fig. 3B,C).

Assessment of symptom remission. The effectiveness of soil solarization in symptom remission or recovery of Verticillium-affected olive trees was evaluated by assessing disease incidence of treated and untreated control trees in July 1985, January 1986, July 1986, and January 1987. With solarization, 62–87% of trees recovered, whereas with natural recovery, only up to 50% of untreated trees recovered for trees with the same initial disease rating of 1 (Fig. 4). The distribution of disease severity among all four categories of the disease scale was greater in untreated trees, compared with the restricted symptom development in solarized trees. Although the percentage of recovery attributable to solarization (47.0–58.8) did not exceed ($P = 0.05$) natural recovery (45.5–61.4) of the untreated trees, disease severity in unsolarized trees was more pronounced

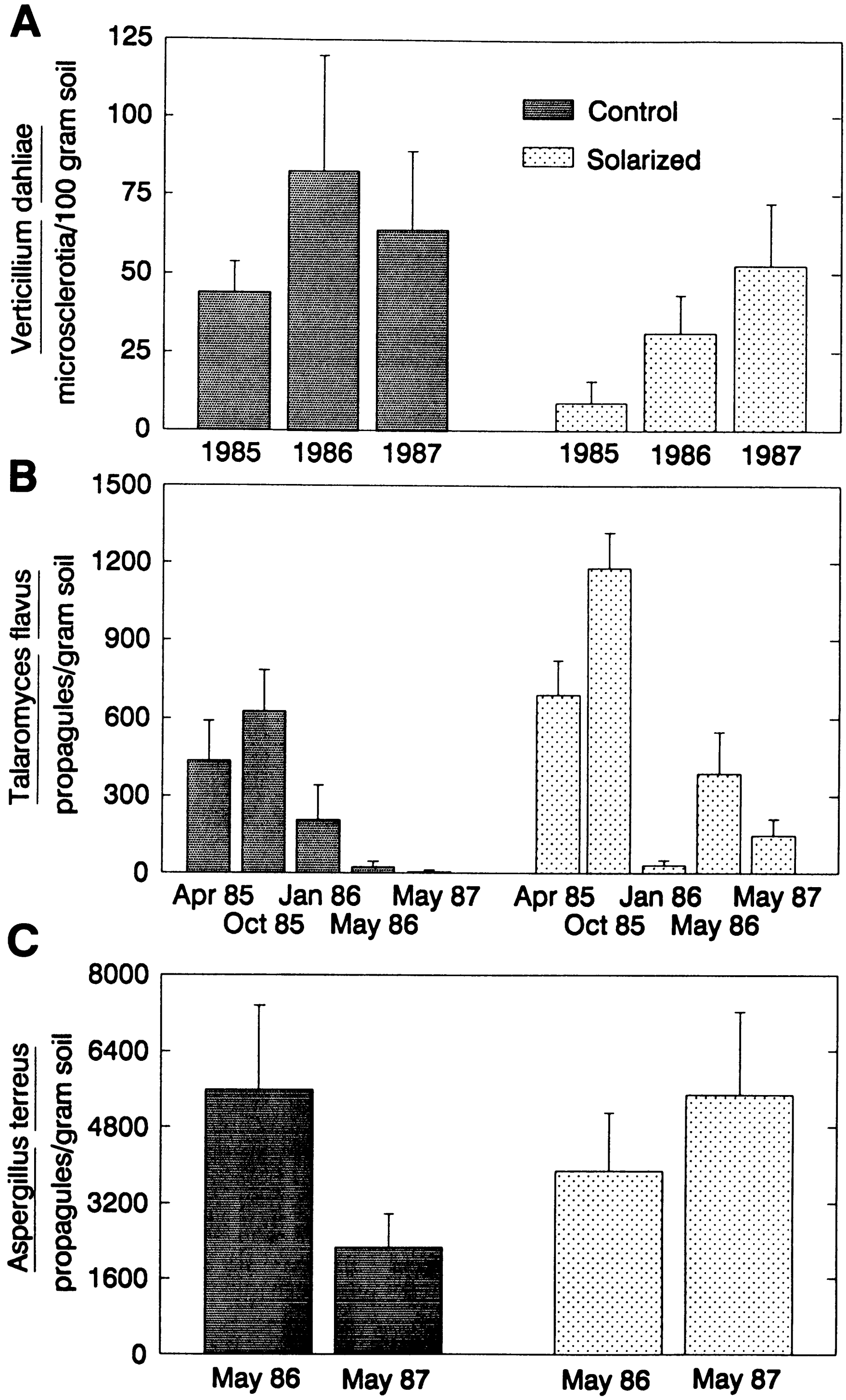

Fig. 3. Effect of soil solarization on the survival and fluctuation of natural populations of **(A)** microsclerotia of *Verticillium dahliae* during three successive years after a single application of the method in comparison with untreated control trees, **(B)** *Talaromyces flavus* in the rhizosphere soil of 10 olive trees in comparison with the fluctuation of the antagonist in 10 untreated control trees, and **(C)** *Aspergillus terreus* in the rhizosphere soil of 10 olive trees in comparison with the fluctuation of natural populations of the fungus in 10 untreated control trees. Histograms represent mean values obtained from nine petri dishes per soil sample from 10 trees per treatment at **(B)** five dates of sampling (April 1985, October 1985, January 1986, May 1986, and May 1987) and **(C)** two dates of sampling (May 1986 and May 1987). The effects for treatment, date of application and treatment × date interaction were significant at $P < 0.02$ in **(A)** and $P < 0.03$ in **(C)**.

and occurred over a greater range of disease classes compared with the solarized trees for trees with an initial disease index of 2 (Fig. 4). Both recovery and symptom remission were higher in solarized (30–70%) trees than in untreated trees (37.5–50.0%), with an initial disease index of 3 (Fig. 4).

A chi-square analysis was applied to the data. Each category (different disease indices) was compared along with the different groups (recovered, mild, intermediate, severe, and dead) for each date (July 1985, January 1986, July 1986, and January 1987). The beneficial effect of individual soil solarization was expressed both as an increased recovery and symptom remission in three successive growing seasons. Mean symptom remission, based on the chi-square analysis, was significantly greater only for trees initially in disease category 1 in solarized soil, compared with remission of symptoms in trees in unsolarized soil. The mean numerical values for a 3-yr period and the final percentages of recovered or diseased trees are summarized in Table 2.

DISCUSSION

A practical and effective approach to control Verticillium wilt in established orchards of susceptible trees remains a remote hope. This is particularly true for the perennial crops that harbor the pathogen in the xylem, such as in the case of *V. dahliae*. Early reports (16,27) concerning inactivation or disappearance of *V. dahliae* in the annual ring of olive and apricot trees, respectively, constituted promising evidence toward the solution of the problem. Indeed, if these reports are true, it could mean that reinfestation of the root system is a prerequisite for the development of new symptoms. Thus, the phenomenon of natural recovery of Verticillium-affected olive trees, also reported by other workers (17), could be primarily attributed to the fluctuation of populations of *V. dahliae* and to the possible antagonistic activity of the soil microflora. If pathogen propagules are eliminated and/or fungal antagonists are surviving

Table 1. Effect of individual soil solarization of olive trees on the survival and increase of natural populations of *Talaromyces flavus*

		Population of *T. flavus* (propagules/g of soil)					
		Healthy trees			Diseased trees		
Treatment	Tree no.	January 1986	May 1986	May 1987	January 1986	May 1986	May 1987
Soil solarization	1	12[a]	637	···	25	500	25
	2	···	500	60	···	212	50
	3	···	212	325	75	···	25
	4	···	925	···	87	375	···
	5	50	1,175	200	12	737	12
	6	25	687	36	···	1,037	160
	7	25	···	···	38	38	···
	8	···	12	70	···	···	120
	9	250	12	···	100	···	···
	10	112	···	12	128	···	···
	11	···	50	84	38	25	···
	12	88	38	700	1,438	87	48
	13	100	12	···	63	3,025	250
	14	63	187	36	50	75	48
	15	···	12	230	···	350	100
Untreated control	1	12	···	···	···	50	···
	2	25	···	···	37	275	···
	3	···	12	···	···	···	150
	4	25	···	···	12	···	···
	5	···	···	···	···	···	···
	6	···	···	···	···	···	···
	7	···	···	···	···	···	···
	8	···	···	···	···	···	···
	9	···	···	···	···	···	···
	10	···	···	···	···	···	···
	11	···	···	···	···	···	···
	12	···	87	···	···	762	···
	13	12	162	72	···	···	···
	14	25	···	···	···	···	···
	15	100	···	48	···	···	···

[a] Values are means of colonies obtained from nine petri dishes per soil sample. Because of the wide range of figures obtained for trees receiving the same treatment, mean values per tree are given separately.

Table 2. Incidence of Verticillium wilt in olive trees within a 3-yr period after a single application of soil solarization

		Olive trees (%) in disease categories[b]									
		0		1		2		3		4	
Treatment	Initial disease index[a]	Mean	Final	Mean	Final	Mean	Final	Mean	Final	Mean	Final
Individual soil solarization	1 [30]	76.0	86.7	18.2	10.0	5.8	3.3	0.0	0.0	0.0	0.0
	2 [17]	53.0	53.0	28.0	17.6	16.1	17.6	2.9	11.8	0.0	0.0
	3 [10]	60.0	70.0	22.5	10.0	12.5	10.0	5.0	0.0	0.0	0.0
Untreated control	1 [58]	49.6	51.7	33.0	24.2	9.0	10.4	4.5	1.7	5.2	12.0
	2 [44]	55.7	61.4	24.5	20.5	14.2	13.6	2.2	0.0	3.4	4.5
	3 [16]	42.2	37.5	12.5	12.5	17.1	25.0	9.3	6.3	18.7	18.7

[a] Rated in July 1984. Based on disease index where 0 = tree healthy, 1 = mild, 2 = intermediate, 3 = severe symptoms, and 4 = tree nearly dead to dead, recorded before the application of the method. Numbers in brackets are the total number of treated or untreated trees per disease index.

[b] Mean percentage of recovered or diseased olive trees obtained in July 1985, January 1986, July 1986, and January 1987. Final percentage obtained in January 1987.

selectively and increasing in the rhizosphere soil, recovery or symptom remission should be expected in the trees growing in soil treated with solarization.

Microsclerotia of *V. dahliae* are vulnerable to soil solarization (13,19,22). Destruction of microsclerotia of *V. dahliae* occurred in fully solarized soils of pistachio nut groves in California (1). Preliminary evidence suggested very satisfactory recovery of olive trees from Verticillium wilt after soil solarization of individual trees (19). Our data verify these earlier findings. Because our results are from a large-scale experiment, it is apparent that natural populations of microsclerotia of *V. dahliae* could be drastically reduced or eliminated in soil of individually solarized olive trees. In contrast to the solarized soil, the untreated soil showed rather constant populations of *V. dahliae* throughout the 3-yr trial. The significant reduction of populations of microsclerotia lasted for at least 2 yr and populations remained lower even in the third year after a single application of the method. Comparison of olive data with findings in artichoke, another perennial host susceptible to *V. dahliae*, shows that soil solarization before the establishment of the artichoke plantation has a pronounced and durable effect on the elimination of fungal propagules and on the prevention of soil reinfestation (22). A long-term effect of soil solarization has been reported for Fusarium wilt of cotton (9). Similarly, a more pronounced effect of soil solarization was shown to exist as a pretreatment of peach orchards (15).

In olive orchards, it is possible that microsclerotia of *V. dahliae* hidden in soil pockets in the north-shaded part of the tree could escape the effects of solarization, be revitalized, and contribute to the increase of the populations and to the reinfection of the trees. It is also possible that olive leaves from diseased olive twigs might eventually contribute to the reinfestation of the groves because leaf petioles and laminae are colonized by *V. dahliae* (21). The partial inefficiency of soil solarization in fully destroying the fungus in shaded areas of the soil could account for the gradual increase of populations of microsclerotia during the third year after soil solarization. In the long run, involvement of hosts susceptible to *V. dahliae* (18) could be prevented from contributing to the increase of the population with chemical control of weeds.

Application of soil solarization to individual trees resulted in a more pronounced recovery in those trees with initially mild symptoms of Verticillium wilt. A tendency for symptom remission was also evident in trees with initially intermediate or severe symptoms. Both recovery and symptom remission exceed natural recovery, which occurred in a significantly lower amount than with the solarized trees. In fact, when the initial disease index is low (mild symptoms), soil solarization has a statistically significant effect on the recovery of the trees ($P = 0.001$). At higher initial disease index, there was no statistical significance between treatment and control. Finally, soil solarization as an overall treatment had a significant effect on the trees ($P = 0.01$). Our findings are also confirmed by Wicks (24) working on the control of *Phytophthora cambivora*

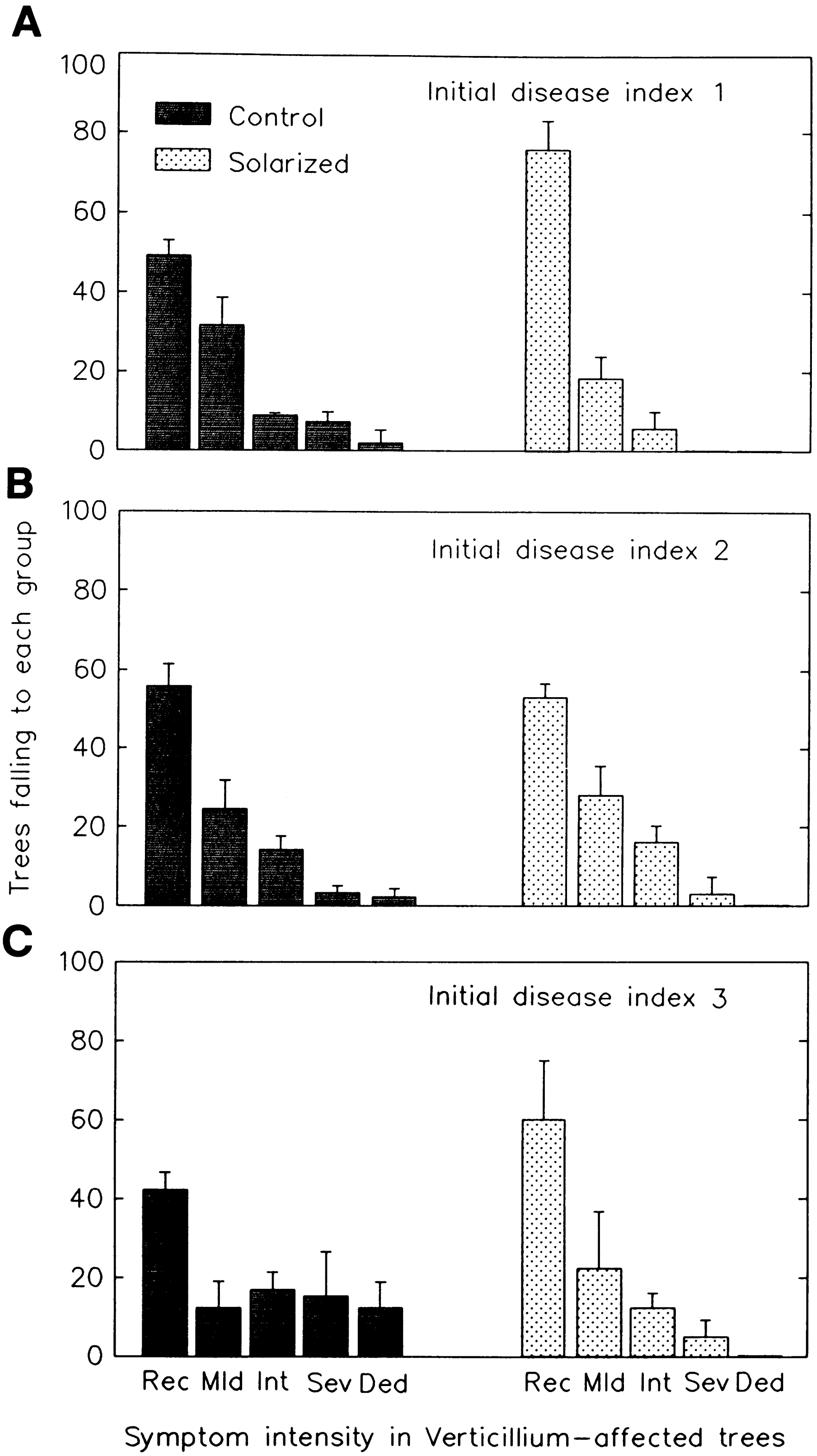

Fig. 4. Effect of soil solarization on the final (January 1987) distribution of trees according to symptom development expressed as percentage of trees falling to each disease index (recovered, mildly, intermediately, or severely affected or dead). The histograms correspond to the mean values obtained from **(A)** 30 solarized and 58 untreated control trees, **(B)** 17 solarized and 44 untreated control trees, and **(C)** 10 solarized and 16 untreated control trees designated as severely affected before the application of the method (July 1984).

(Petri) Buisman of almond and cherries after the application of soil solarization. It was unfortunate that frost damage in the experimental area in March 1987 masked foliar symptoms and prevented any further evaluation of the method.

Because *V. dahliae* is almost always able to infect the root system, with the exception of the hot summer and cold winter months, treated trees could already be infected before the application of solarization in July. Thus, the overall beneficial effect of solarization could be limited. It is evident, however, that recovery or symptom remission figures reflect a definite beneficial and long-term effect of the treatment. Soil solarization had a potential for long-term control of Verticillium wilt of pistachios for 12–18 mo after soil mulching (1) and may have the same potential for use in olives.

The involvement of heat-resistant antagonists of *V. dahliae* in the long-range effect of soil solarization on globe artichokes has already been postulated (22). *T. flavus*, a known antagonist of *V. dahliae* (11,22), and *A. terreus*, a potential antagonist of the pathogen (22), have been found to occur, survive solarization, and increase in the soil of trees treated with solarization compared with that for untreated control trees. The populations detected for both antagonists were significantly higher in soil of treated than of untreated trees. Numerical differences in populations of *V. dahliae* could mean that *T. flavus* could be partially involved in delaying increase of *V. dahliae* and prolonging the effectiveness of the method. The biological effect of solarization (8,15,22) should be effective in the described areas because the application of the method is restricted in a small soil area. Other fungal or bacterial antagonists may contribute to the longevity of the effect (2).

Our data clearly demonstrate that individual soil solarization could be a practical and economical measure for the control of Verticillium wilt in irrigated olive orchards. This effect could be further exploited by earlier applications of the technique (May) and combining solarization with the addition of biological preparations of fungal or bacterial antagonists to *V. dahliae*.

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