

Effect of Soil Solarization on Populations of Selected Soilborne Microorganisms and Growth of Deciduous Fruit Tree Seedlings

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ABSTRACT

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Two fallowed field sites of differing soil type in the San Joaquin and Sacramento valleys in California, were treated by soil solarization (covering of moist soil with clear polyethylene sheeting to achieve high soil temperatures) for 4.5 wk in the summer. Soil samples from solarized and nonsolarized plots were periodically collected and assayed with selective media for population densities of certain microorganisms. Plots were planted with English walnut and cultivar Nemaguard peach seeds after solarization. Population densities of *Agrobacterium* spp., fluorescent pseudomonads, Gram-positive bacteria, and fungi recoverable on PDA were greatly reduced immediately following solarization. Actinomycetes and thermophilic/thermotolerant fungi were affected to a lesser extent.

Additional key words: solar heating, soil pasteurization, biological control.

Agrobacterium spp. and populations of Gram-positive bacteria remained significantly depressed in solarized soil after 6-12 mo. Fluorescent pseudomonads and "total" fungi quickly recolonized the treated soil, while actinomycetes and thermophilic/thermotolerant fungi attained higher population densities following soil solarization. After one growing season, there were no detectable differences in stand counts of peach or walnut seedlings. However, peach seedlings grown in solarized soil showed increases in plant height of 25% and fresh weight of 42%, and walnut seedlings showed increases in height and fresh weight of 26 and 58%, respectively, compared with those grown in nonsolarized soil.

Soil solarization is a term used to describe hydro/thermal soil heating accomplished by covering moist soil with clear polyethylene sheeting during the summer months. Several workers reported the success of this treatment in reducing plant disease caused by soilborne pathogens (3-5,8,9,10,14). In addition to reduced disease incidence and severity, increased plant growth and crop yields were observed following the treatment (2,4,5,9,10,15). However, previous studies reporting reduced population densities of soilborne pathogens were confined to target organisms, and did not determine the effect of solarization on a broader range of soil microflora, including those that may be antagonistic to plant pathogens. In this study, population densities of several groups and genera of soilborne microorganisms in solarized and nonsolarized soil were periodically estimated during the 12-mo period following soil solarization to determine the initial and residual effects of the treatment on a wide spectrum of organisms. In addition, soil analyses were made to determine the effect of the treatment on the nutritional status of the soils. Also, the growth of walnut and peach seedlings, planted following soil solarization, was recorded. A report on a preliminary study was published (19).

MATERIALS AND METHODS

Experimental design and soil sampling. Field experiments were done near Davis, Hickman, Winton, and Atwater, CA. Effective plot sizes were a minimum of 268 m². The Davis site, on Yolo fine sandy loam soil with some clay strata (heavy soil), was solarized 2 mo after removal of a 20-yr-old walnut orchard. The plot was preirrigated with 12-15 cm of water 8-10 days prior to covering soil with clear polyethylene sheeting 0.025 mm (1 mil) thick. Following covering, an additional 2 cm of water was applied under the plastic.

The Hickman site was in a deciduous tree-fruit nursery on Hanford sandy loam soil (light soil) that had been fallowed for 6 mo following the removal of apple and cherry trees. The plot was sprinkler-irrigated with 2 cm of water the night before the polyethylene sheeting was applied. The Winton and Atwater sites were planted to deciduous fruit trees on peach rootstocks. The soil type of both orchards was Atwater sand. Orchard plots were covered approximately 2-4 days after routine irrigation by the orchard operators. Irrigation water applied at each of the sites was sufficient to wet the soil throughout the 0-46 cm sampling depth to above field capacity, except at the orchard sites, where the tarps were applied to soil that had dried to below field capacity near the soil surface. The tarps were removed after 4-4.5 wk of treatment at all sites. The experimental design was randomized single-block with five replications for both the solarized and control treatments, except in orchard plots, where 10 randomly selected trees were used for solarized and 10 for nonsolarized treatments. Recording-thermograph sensors were buried at 15 and 30 cm at both sites in one replication of each treatment.

Following removal of the plastic tarps, soil samples were taken with a standard 1.6-cm (inside diameter) core auger. For all samples, eight to 10 cores were collected per replication and bulked by three depth ranges (0-15, 15-30, and 30-46 cm). All soil samples were taken shortly after an irrigation, except at the orchard sites. At the Davis site, samples were collected at intervals during the year following treatment. Due to grower requirements, the Hickman site was abandoned after 6 mo and only one subsequent sampling was made. No further samples were taken at the orchard sites, as the first samples were collected for soil properties analysis only.

Culture media. Six selective media were used to estimate and compare population densities of soil microorganisms: potato-dextrose agar (PDA), acidified with five drops per 100 ml 25% lactic acid (21), and including 250 µg of streptomycin sulfate per milliliter added after autoclaving to inhibit bacterial growth, was used for "total" fungi; yeast-glucose (YG) agar (20), amended with lactic acid and streptomycin sulfate as above, was used for thermophilic/thermotolerant fungi; Schroth's agar (17) was used for *Agrobacterium* spp.; King's B agar (11) (as modified by Sands

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and Rovira [16]) was used for fluorescent pseudomonads; water agar at pH 10.5 (6) was used for "total" actinomycetes; and 523 agar (7), modified with 2 μg of sodium azide, 32 μg of polymixin B sulfate, and 250 μg cycloheximide per milliliter, was used for "total" Gram-positive bacteria.

Assay procedure for counts of soil microorganisms. From each bulked sample, two 1-g subsamples were each added to 10 ml of distilled water (pH 7.2), shaken for 2 min on a vortex mixer at "fast" speed, allowed to settle for 1 min, and diluted into water blanks. At the proper dilution for colony counting, 0.1 ml of the suspension (volume equivalent of 10 mg of soil per plate and sensitivity no greater than 100 colony-forming units per gram of soil) was spread on the various agar media with an L-shaped rod. Normally, aliquots from each of the 1-g subsamples were introduced into four plates of each medium.

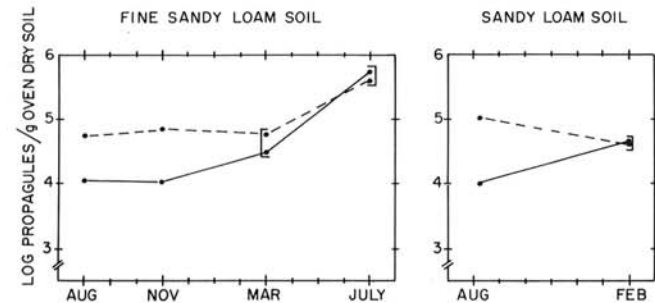


Fig. 1. Effect of soil solarization on population densities of "total" fungi in soil (0-46 cm depth) at two field sites (1979-1980). Brackets ([,]) indicate data points not significantly different ($P < 0.05$) according to Student's t -test of independent means. Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments.

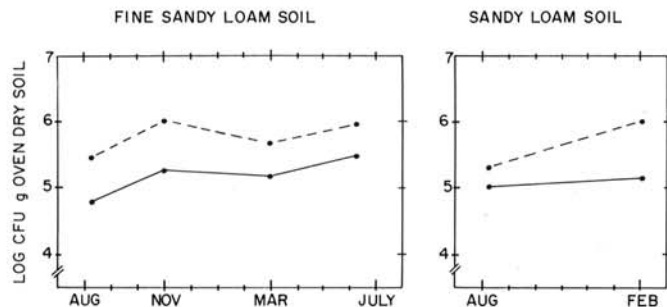


Fig. 2. Effect of soil solarization on population densities of Gram-positive bacteria in soil (0-46 cm depth) at two field sites (1979-1980). Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments. CFU means colony-forming units.

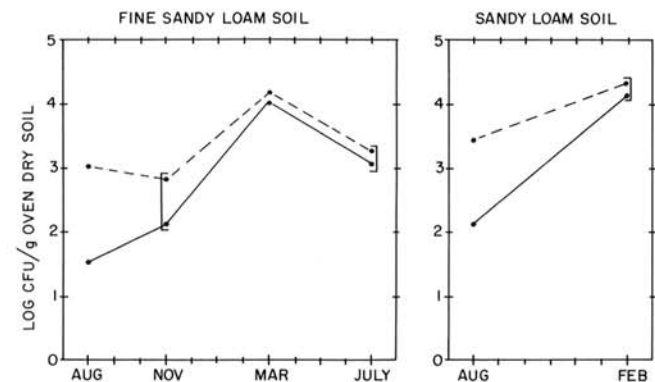


Fig. 3. Effect of soil solarization on population densities of fluorescent pseudomonads in soil (0-46 cm depth) at two field sites (1979-1980). Brackets ([,]) indicate data points not significantly different ($P < 0.05$) according to Student's t -test of independent means. Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments. CFU means colony-forming units.

The plates were incubated at 28 C (except those incubated at 46 C on YG medium). Bacterial colonies were counted after 3-5 days of incubation, fungal colonies after 8-10 days, and colonies of actinomycetes after 14-21 days.

Soil properties assay. Wet, bulked soil (0-46 cm depth) from the first posttreatment samples at all sites was analyzed for mineral nutrients and other properties approximately 2-4 mo after collection by the Cooperative Extension Soils Laboratory, Department of Land, Air, and Water Resources, University of California, Davis 95616.

Plant growth analyses. Following soil solarization, English walnut (*Juglans regia* L.) and peach (*Prunus persica* (L.) Batsch 'Nemaguard') were planted as indicator nursery crops at the Davis site (two rows each per replication). Stand counts of peach seedlings were recorded in December, and walnut seedlings in July. In September (11 mo after planting), trees were removed by undercutting approximately 30 cm below the soil surface, and again stand counts were made. Thirty randomly picked seedlings of each species per replication were measured for plant height (from the soil line to the uppermost growing point) and fresh weight. Due to termination of the Hickman plot, no indicator plants were grown at the site. However, treated and untreated soil (fallow for 6 mo following solarization) was brought to the greenhouse and planted to radish and cotton.

RESULTS

Assay of soil microorganisms. During the 4- to 4.5-wk treatment periods, soil temperature at the 15-cm depth reached 49 C under the tarps at the Hickman site (10 C higher than nonsolarized soil at 15 cm), 46 C at the Davis site (7 C higher than in nonsolarized soil), and 45 C at the Atwater site (8 C higher than in nonsolarized soil).

Immediately after removal of the plastic tarps, overall population densities, in solarized soil as compared to control soil, of "total" fungi were reduced 90 and 85% at Davis and Hickman,

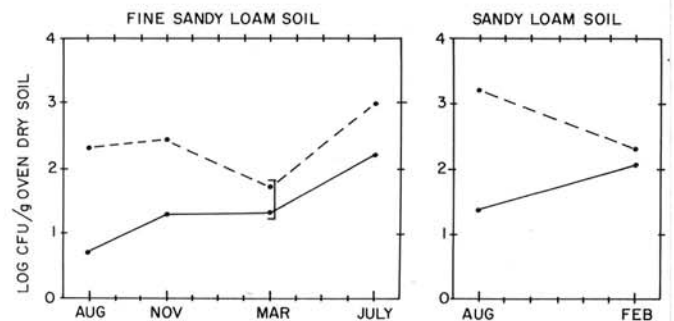


Fig. 4. Effect of soil solarization on population densities of *Agrobacterium* spp. in soil (0-46 cm depth) at two field sites (1979-1980). The bracket (]) indicates data points not significantly different ($P < 0.05$) according to Student's t -test of independent means. Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments. CFU means colony-forming units.

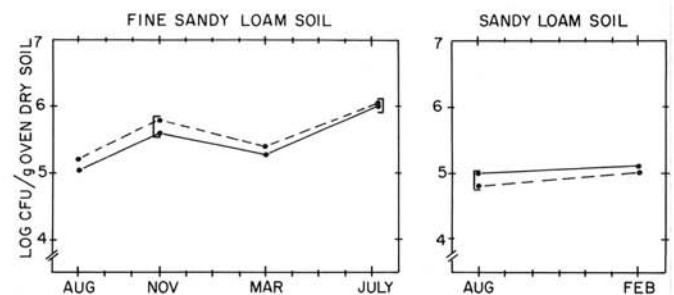


Fig. 5. Effect of soil solarization on populations of actinomycetes in soil (0-46 cm depth) at two field sites (1979-1980). Brackets ([,]) indicate data points not significantly different ($P < 0.05$) according to Student's t -test of independent means. Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments. CFU means colony-forming units.

respectively (Fig. 1); "total" Gram-positive bacteria by 84 and 69% (Fig. 2); fluorescent pseudomonads by 96 and 94% (Fig. 3); and *Agrobacterium* spp. by 98 and 98% (Fig. 4), respectively. "Total" actinomycetes (Fig. 5) and thermophilic/thermotolerant fungi (Fig. 6) reacted differently at the two sites, showing decreases in population at the Davis site (fine sandy loam soil) and no significant differences at the Hickman site (sandy loam soil). In subsequent samplings at both sites, population densities of *Agrobacterium* spp. and Gram-positive bacteria remained significantly depressed ($P < 0.05$) in the treated soil compared to the control soil. The population densities of "total" fungi and fluorescent pseudomonads, although initially greatly depressed in the treated soil, rapidly recolonized the soil so that there were no significant differences between the treatments after 3–7 mo. Thermophilic/thermotolerant fungi increased in the solarized soil to significantly higher numbers than in the untreated soil by the end of the evaluation period. Counts of colony-forming units of actinomycetes also increased in the treated soil, and were either higher than, or did not differ from, those in the control soil 3–6 mo after completion of the treatment. In general, the greatest effect of the treatment was in the upper 15 cm of soil, and few significant changes in microbial population densities occurred in the 30–46 cm depth range, except for actinomycetes and thermophilic/thermotolerant fungi, which increased in the Davis soil.

Soil properties analysis. In the Hickman plot (Table 1), the solarized soil contained lower concentrations of available phosphorus, potassium available in exchangeable phase, water-extractable nitrate-nitrogen, ammonium-nitrogen available in exchangeable phase, and water-soluble calcium than did the untreated soil. Increased amounts of available zinc and water-soluble magnesium were detected. At Davis, on the other hand, solarized soil contained higher levels of phosphorus, potassium, and nitrate-nitrogen, while concentrations of zinc, ammonium-nitrogen, calcium, and magnesium were decreased, compared to those of the nonsolarized soil. In the orchard soils, again, there were no consistent changes, except for increased amounts of nitrate-nitrogen, and decreased phosphorus, calcium, and magnesium at both orchard sites.

Plant growth analyses. Comparisons of stand counts 1–3 mo after emergence of seedlings taken at the Davis site showed statistically significant ($P < 0.05$) increases in surviving peach (32%) and walnut (47%) seedlings growing in soil that had been solarized compared to control soil. By the end of the growing season, however, no significant differences were apparent in the stand count of either of the crops when solarized and nonsolarized treatments were compared. Mean peach tree height was increased by 24.7%, and walnut height by 26.1% in covered treatments, compared with nonsolarized soil treatments. Similarly, mean fresh weight was increased by 42.4% in peach, and 58.1% in walnut trees grown in solarized soil compared with nonsolarized soil. Increased growth was not solely attributable to earlier seed germination in solarized plots. Although no measurements were taken immediately following emergence, height differences in plants

grown in solarized and nonsolarized soil were easily visible in the field throughout the observation period. All harvested plants were apparently disease-free, except for a minor incidence of crown gall in those grown in nonsolarized soil (18). Plant growth data at the Davis site are summarized in Table 2. The cotton and radish seedlings grown in the Hickman soil in the greenhouse showed increases in percentages of both emergence and plant height as a result of solarization similar to those that were observed in the Davis field plot.

DISCUSSION

Previous studies on the effect of soil solarization on target plant pathogenic fungi and bacteria reported reductions of nearly 100% immediately following the treatment (5,9,15). Evidence presented here indicates that population densities of actinomycetes and thermophilic/thermotolerant fungi remained relatively high following solarization, and that these increased to levels higher than present in untreated soil.

The fungi most often isolated from soil by the method used in this study were thermotolerant *Aspergillus* and *Penicillium* spp., which produce large numbers of small, light-weight conidia. These results are consistent with previous reports (22), and especially with those concerning the effects of soil steaming (12). *Bacillus* spp. were the predominant Gram-positive bacteria surviving soil solarization.

Work done in Israel (2) reported increased amounts of soluble mineral nutrients in the upper 10 cm of soil following solarization. However, for soils used in the present study (0–46 cm depth), few consistent changes were found. Although increases in nitrate-nitrogen were found in three of the four soils tested after solarization, major differences in nutrient levels were not apparent. Differences between the results obtained in Israel and those from California may have been due to differences in assay procedure (soil depths tested), soil types, previous fertilization, or cropping

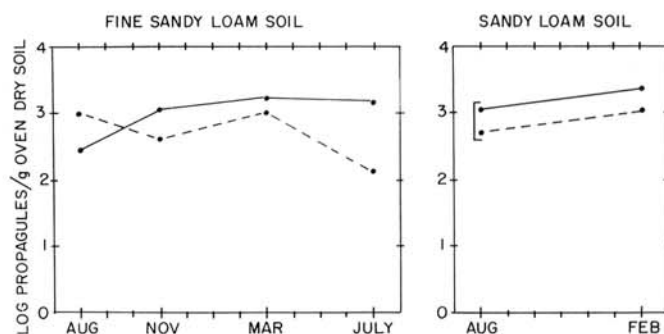


Fig. 6. Effect of soil solarization on populations of thermophilic/thermotolerant fungi in soil (0–46 cm depth) at two field sites (1979–1980). The bracket ([]) indicates data points not significantly different ($P < 0.05$) according to Student's *t*-test of independent means. Solid lines indicate solarized treatments; broken lines indicate nonsolarized treatments.

TABLE 1. Soil properties analysis^a following soil solarization at four field sites in California

Year and site	Treatment	Moisture ^b (%)	pH ^c	Conductivity (mmhos/cm)	P (μg/g)	K (μg/g)	Zn (μg/g)	NO ₃ -N (μg/g)	NH ₄ -N (μg/g)	Ca (me/L)	Mg (me/L)
1979											
Hickman	Solarized	21	5.8	0.87	20.0	124	2.9	17.2	3.2	2.5	1.9
	Control	21	5.8	0.95	23.0	139	2.2	21.1	4.4	2.8	1.7
Davis	Solarized	38	6.9	0.61	13.9	250	0.45	16.2	3.2	1.5	3.7
	Control	39	6.9	0.62	12.8	225	0.53	15.0	4.4	1.6	4.1
1980											
Winton	Solarized	nd ^d	5.6	nd	13.7	50	6.4	6.7	2.2	1.0	0.3
	Control	nd	5.3	nd	14.4	50	4.7	4.8	3.6	1.1	0.4
Atwater	Solarized	nd	5.6	nd	24.0	47	6.8	11.6	4.4	1.2	0.3
	Control	nd	5.0	nd	39.0	52	7.6	3.7	4.0	1.4	0.6

^aData supplied by Cooperative Extension Soils Laboratory, Department of Land, Air, and Water Resources, University of California, Davis 95616.

^bSaturation percentage.

^cMeasured in saturated paste.

^dnd = No data.

TABLE 2. Effect of soil solarization on survival and growth of peach and walnut seedlings at Davis, CA in 1979

Crop	Soil treatment	Surviving seedlings	Surviving seedlings	Fresh wt. (g) ^a	Height (cm) ^a
Nemaguard peach	Solarized	2,025** ^{b,c}	2,204 ^c	435.6** ^c	160.1** ^c
	Control	1,531 ^b	2,100 ^c	305.9 ^c	128.4 ^c
English walnut	Solarized	529* ^d	853 ^c	157.3** ^c	21.2** ^c
	Control	360 ^d	670 ^c	99.5 ^c	16.8 ^c

^a Means for 150 randomly sampled seedlings.

^b Data taken 1 mo after emergence.

^c Values with single asterisks (*) are significantly different at ($P < 0.05$), and with double asterisks (**) at ($P < 0.01$) according to Student's *t*-test of independent means.

^d Data taken 2 mo after emergence.

^e Data taken 11 mo after emergence.

history.

The enhanced plant growth responses in solarized soils reported here have been characteristically observed when solarized and nonsolarized soils were compared in other studies.

The limited changes in nutrient levels as determined by soil chemical analyses, and the survival of relatively high populations of certain bacteria and fungi, suggested that the increased plant growth response following solarization may be due to changes in populations of soil microorganisms. This possibility has been raised in previous reports on the effectiveness of soil solarization (3-5,8,9). The data presented here support this theory, in that soil microorganisms likely to be beneficial to plant growth are either less affected by soil solarization (eg, *Bacillus* spp., actinomycetes) than are pathogenic organisms, or they are able to quickly recolonize treated soil (eg, fluorescent pseudomonads).

The effect of selective soil disinfestation, ie, killing pathogens and leaving competitive saprophytes (spore-forming bacteria and actinomycetes) by aerated steam, is well described (1,12,13). Soil solarization may operate similarly, except that maximum temperatures achieved are lower and the time of treatment is longer. Solarization is a milder treatment than aerated steam, and in some instances, may only injure or weaken pathogens rather than kill them (3,4,9). Thermophilic/thermotolerant fungi, as well as the spore-forming bacteria and actinomycetes appear to flourish after the treatment. Large decreases in population densities of microorganisms assayed immediately following tarping were frequently noted in this study. However, substrates made available by the effects of solarization were apparently, in most cases, rapidly recolonized by surviving microorganisms beneficial to plant growth and/or antagonistic toward pathogenic organisms. Although the resulting microcommunity may not represent a climax state in the soil, there is evidence (15) that disease reduction in solarized soils may last at least two growing seasons.

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