Suppression of Major and Minor Pathogens by Fluorescent Pseudomonads in Solarized and Nonsolarized Soils

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ABSTRACT

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Strains of fluorescent pseudomonads increased the growth of tomato plants in nonsolarized soil but not in solarized soil. Vital staining showed that fluorescent pseudomonads colonize the root cortex and, apparently, internally colonize the younger lateral roots of artificially inoculated plants growing in nonsolarized soil. The minor pathogen *Penicillium pinophilum* caused growth retardation of tomato and cotton plants. It colonized tomato roots in nonsolarized, methyl bromide-fumigated, and solarized soils (to a much lesser extent). Inoculation of tomato roots with fluorescent pseudomonads or transplanting tomato roots to solarized soil suppressed colonization by *Penicillium pinophilum* and nullified plant-growth retardation. Inoculation of plant roots with plant growth-promoting strains of *Pseudomonas putida, P. fluorescens,* and *P. alcaligenes* reduced the incidence of disease caused by *Sclerotium rolfsii* in bean, Fusarium wilt in cotton and tomato, and reduced colonization of cotton roots by pathogens that cause these diseases. Induced suppressiveness against *S. rolfsii* in bean and *Fusarium oxysporum* f. sp. *vasinfectum* in cotton was observed in solarized soil. We suggest that fluorescent pseudomonads are effective agents in suppressing both major and minor pathogens in soil.

Additional keywords: beneficial microorganisms, deleterious microorganisms, melon, plant growth-promoting rhizobacteria.

The increased growth response of plants in solarized soil is a well-documented phenomenon, shown in the absence of known pathogens, and verified both in greenhouse experiments and under field conditions (1,7,8,15,16,26,27). This response is attributed to various chemical, physical, and biotic factors (1,8,16,27). Pronounced changes in microbial activity occur in soil during and following solarization, indicating that soil microorganisms play an important role in the increased growth-response phenomenon

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(1,8,10,13,27). Frequently, solarized soils become suppressive toward pathogens (10,11,14), while fluorescent pseudomonads increasingly colonize the rhizosphere and roots following solarization of soil or container media (7,8). Strains of these bacteria improve growth of inoculated plants in the greenhouse. In contrast, the establishment of fungi in the rhizosphere and roots following solarization is suppressed (8). Plant growth-promoting pseudomonads are thought to act through direct antagonism of major pathogens, through antibiotic production, through competition with pathogens for essential nutrients such as iron, or, more directly, through plant-growth promotion (2,4,17,23–25). Some studies also have suggested that fluorescent pseudomonads may stimulate plant growth through suppression of deleterious microorganisms and minor pathogens (23,24,28). Certain fungal species such as *Penicillium pinophilum* Hedgc. suppress plant growth and can be regarded as minor pathogens (8,22,30) because they supress growth without inducing typical disease symptoms such as root rot or wilt. Many studies have dealt with the interaction between fluorescent pseudomonads and major pathogens in order to evaluate the former's potential as biocontrol agents (4,17,23–25), but much less is known about the interaction of these bacteria with minor pathogens. In the present study, we investigated the potential of fluorescent-pseudomonad strains to suppress minor and major pathogens and the suppressive or conducive nature of solarized and fumigated soils toward minor and major pathogens.

MATERIALS AND METHODS

Soil. Soil was collected from a field at Rehovot, Israel (3.8% clay, 0.0% silt, 96.2% sand, and 0.4% organic matter; pH 6.9). Soil was untreated, solarized, or fumigated with methyl bromide in experimental field plots (8 × 16 m) during 1988–1990. Soil samples were collected from the upper 20 cm (after removal of the top 2–3 cm) of untreated, solarized, or methyl bromide-fumigated soils and were kept in containers in a shaded place until use. Solarization was carried out manually using transparent polyethylene sheets (40–50 μm thick) to mulch preirrigated soil for 6 wk during July and August 1988–1990 (8,9). Typical maximal temperatures of the solarized soils at depths of 10 and 30 cm were 45–48 and 39–41 C, respectively. Temperatures of the corresponding nonsolarized soils were 7–12 C lower. In September, fumigation with methyl bromide, at a rate of 55 g per square meter, was carried out by applying the hot-gas technique (using commercial equipment).

Plants. Tomato (*Lycopersicon esculentum* Mill. 'Rehovot 13'), cotton (*Gossypium barbadense* L. 'Pima S-5'), bean (*Phaseolus vulgaris* L. 'Mangold'), and melon (*Cucumis melo* L. 'En-Dor') were used in these experiments.

Culture media. King's B agar (KB) (3) was used for culturing *Pseudomonas putida*. KB modified by the addition per liter of 100 mg of cycloheximide, 50 mg of ampicillin, 12.5 mg of chloramphenicol, and supplemented with 5 mg of pentachloronitrobenzene (PCNB) to suppress *Rhizopus* spp. (8) was used for enumeration of fluorescent pseudomonads from soil and root parts. Potato-dextrose agar (PDA) was used for culturing *Penicillium pinophilum*. Martin's agar (3) supplemented per liter with 5 mg of PCNB (8) was used for enumeration of *Penicillium pinophilum*. Peptone-PCNB medium (8) acidified with 1 ml of 90% lactic acid per liter and supplemented per liter with 250 mg of chloramphenicol, instead of streptomycin, was used for the isolation and enumeration of Fusarium from soil and root parts. Yeast-extract agar (YEA) was used to culture Fusarium for inoculation tests. Finally, Joham medium (3) was used for culturing *Sclerotium rolfsii* Sacc. for sclerotia production.

Microorganisms and inoculum preparation. Bacterial strains of *Pseudomonas putida, P. fluorescens,* and *P. alcaligenes* originally were isolated from the roots of tomato plants grown in solarized, Rehovot soil (8). Strains of *Pseudomonas putida* were spontaneous, resistant mutants to streptomycin (MS10 and MS2). The mutants were isolated from the roots by plating roots on modified KB supplemented per liter with 100 mg of streptomycin. All strains were defined as plant-growth promoting because they significantly increased dry weight of tomato plants by 35–42% after root inoculation in greenhouse tests (8). Bacterial strains were stored in a nutrient-glycerol medium at −70 C until use. Bacteria were spotted onto KB agar and grown for 24 h. Bacteria were then transferred to KB agar for an additional 48 h, suspended in distilled water containing 0.5 mM of $CaCl_2$ (12), washed, and their concentration was adjusted to 10^8 cells per milliliter as determined by optical density. Bacterial-cell suspensions were used for root inoculation.

Two isolates of *Penicillum pinophilum* initially were isolated from the rhizosphere of tomato plants grown in nonsolarized, Bet HaShitta soil (8,9). They were defined as minor pathogens because they reduced the dry weight of inoculated tomato seedlings in greenhouse tests by 47–58% (8). Fungi were cultured for 8 days on PDA, after which, the conidia were harvested, suspended in distilled water supplemented with 0.1% $MgSO_4{\cdot}7H_2O$, and washed. Final conidial concentration was adjusted $10^2 - 10^6$ conidia per gram of soil.

Fusarium oxysporum Schlechtend. f. sp. *vasinfectum* (Atk.) Snyder & Hans. (race 3), *F. o. melonis* Snyder & Hans. (race 0), and *F. o. lycopersici* (Sacc.) Snyder & Hans. (race 2) were isolated from diseased cotton, melon, and tomato plants, respectively. The pathogens were grown for 8 days on YEA. Inoculum was prepared as previously described for *Penicillium pinophilum*.

Sclerotia of *Sclerotium rolfsii* Sacc. were collected from the soil around the root neck of diseased bean plants and screened to remove soil aggregates by wet sieving. Sclerotia also were obtained by culturing the pathogen on Joham agar for 3 wk, after which the dishes were left open for an additional 3 wk to allow sclerotial drying and maturation.

Inoculation tests. Combined inoculation of plants with bacteria and fungi was conducted. First, cotton, tomato, and melon seedlings were pulled out of soil 1 day after emergence, and their roots were dipped in a suspension of the tested bacterial strains (10^8 cells per milliliter in distilled water supplemented with 0.5 mM of $CaCl_2$) for 10 min (12). Similarly, germinating bean seeds (2 days after placement on wet cotton wool) were dipped in the bacterial suspensions. Subsequently, bacteria-inoculated plants were transplanted in pots (six plants per pot) in soil artificially inoculated with the tested fungi. Fungal inoculations were carried out by mixing washed conidia of either *Penicillium pinophilum, F. o. vasinfectum, F. o. lycopersici,* or *F. o. melonis* with nonsolarized, solarized, or methyl bromide-fumigated Rehovot soils to the desired inoculum density, as indicated. The soils were free of any known pathogens prior to infestation, as assayed by planting the seedlings in soil without any bacterial or fungal infestation. Infestation of the soil by *S. rolfsii* was performed by placing two sclerotia in the soil near each germinating bean seed (six per pot) previously inoculated with bacterial strains as described. In an additional treatment with beans, *S. rolfsii* sclerotia were placed in nylon nets adjacent to germinating bean seeds (either uninoculated or inoculated with bacteria), were retrieved after 1,2, and 4 days, and were washed and placed on filter paper moistened with bromcresol green to be tested for germination (19). All treatments were conducted in six replicates (pots) and arranged in a completely randomized design in a greenhouse (21–27 C). Disease progress caused by the introduced, major pathogens was recorded throughout plant growth. The effect of *Penicillium pinophilum* on plant growth was assayed by uprooting the plants grown in soil infested with the fungus and determining the dry weight of the shoots (8).

Test for antagonism on agar. This test was carried out as previously described (8). Three strains each of *Pseudomonas putida, P. fluorescens,* and *P. alcaligenes*, were cultured on KB agar for 48 h. Bacteria from each strain were then spotted at three equidistant points 1 cm from the edge of petri dishes (85 mm in diameter) containing PDA. After 48 h of growth in the dark at 30 C, mycelial disks of the test fungi were placed in the center of each dish. During further incubation, lasting 6 days in the dark at 30 C, the cultures were followed to detect inhibition

of mycelial growth or lysis. Inhibition was assayed by measuring the distance between the fungal and bacterial colonies and comparing the colonies with a fungal colony in plates without bacteria. Possible lysis of fungal mycelium that reached the bacterial colonies was microscopically inspected.

Microbial colonization of rhizosphere. Inoculated plants were uprooted along with the soil adhering to their roots. Soil adhering to the roots was collected by shaking the roots in sterile tubes. Remaining soil, 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% $MgSO_4 \cdot 7H_2O$. Both soil fractions were combined, constituting the rhizosphere-soil sample. Rhizosphere-soil suspensions were serially diluted, and samples of 0.1 ml (for bacterial counts) or 0.2 ml (for fungal counts) were spread on five petri dishes containing the appropriate selective medium. Dishes were incubated in the dark at 28 C. Colonies were counted after 4–8 days. Results are expressed as colony-forming units (cfu) per gram of soil (dried at 105 C for 48 h).

Microbial colonization on roots and shoots. A direct assay of microbial populations of the whole-root tissue was carried out by macerating washed roots in 0.1% water agar (supplemented with 0.1% $MgSO_4 \cdot 7H_2O$) with a high speed homogenizer (Ultra Turrax, Janke & Kunkel, Germany) for 1 min. The suspension was diluted further, and 0.1–0.2-ml samples from the proper dilution were spread on the appropriate medium. Microbial populations of the interior tissue of roots, hypocotyl or epicotyl, were assayed similarly, except washed roots were surface-disinfested with 0.1% NaOCl for 30 s before maceration.

Vital staining of bacteria in root tissue. Staining was conducted essentially as described by Patriquin and Dobereiner (21). Roots of tomato plants inoculated with bacteria were washed free of soil with distilled water, surface-disinfested with 1% NaOCl for 30 sec, and cut into 5–8 cm segments. These were soaked in small beakers containing potassium phosphate buffer (0.05 M, pH = 7.0), supplemented per liter with 0.625 g of malate and 1.5 g of 2,3,5-triphenytetrazolium chloride, and incubated at room temperature for 14 h. Finally, cross sections of the tissue were cut manually with a razor blade and examined under a light microscope. Live bacteria reduce triphenyltetrazolium chloride to crystalline formazan, which is then trapped within bacterial cells; bacterial aggregates within the root tissues, as a result, are seen as dark violet, while the root tissue is colored pale pink (21).

Statistical analyses. Experiments testing the effect of combined inoculation with bacteria and fungi were conducted in factorial design and analyzed accordingly. Greenhouse experiments were carried out with six pots per treatment serving as the experimental units (replications). All experiments were repeated three times. Separate analysis of each trial showed that the variances of the experimental error among the three trials were homogeneous.

TABLE 1. Effect of fluorescent pseudomonads on the relative dry weight of tomato plants grown for 28 days in nonsolarized or solarized soils

Source of bacteria[b]	Number of strains	Dry weight (% of control)[a] Nonsolarized	Solarized
Control		100	100
Rhizosphere	6	120–165[c]	88–112
Whole-root tissue	12	128–142	92–115
Whole-root tissue (SR)	7	123–153	95–110

[a]Experiment was conducted with six replicates per treatment, each repeated three times. Data shown represent the combined data of the three trials. Figures represent the range of the tested strain means.

[b]Bacteria were originally isolated from tomato plants grown in solarized soil and were defined as plant-growth promoters in a preliminary study. Inoculation was carried out by dipping roots of tomato seedlings in suspensions of tested bacteria, followed by replanting in either nonsolarized or solarized Rehovot soils. SR = streptomycin-resistant.

[c]All bacteria within the indicated bacteria group significantly increased the dry weight of tomato plants compared to the respective control (uninoculated) plants according to Student's *t* test ($P \leq 0.05$).

Therefore, data from repeated experiments were combined. Analysis of the pooled data showed no significant interaction between the treatments and the replicate experiments; thus, only the effect of the treatments (i.e., bacterial and fungal inoculation) is shown in the analyses. Statistical analyses of the results included analysis of variance, Student's *t* test, Fisher's least significant difference test, and calculating standard error, as indicated. Percentages first were transformed to arcsin square roots prior to analyses. Data presented in logarithmic scale also were analyzed in this scale. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC, release 6.04 for personal computer) at $P \leq 0.05$.

RESULTS

Establishment of fluorescent pseudomonads in roots after inoculation. Twenty-five strains of fluorescent pseudomonads that increased the growth of tomato plants in nonsolarized soil in previous experiments (8,9), identified as *Pseudomonas putida*, *P. fluorescens*, and *P. alcaligenes*, were tested in both nonsolarized and solarized soils. In the present study, these bacteria significantly increased the dry weight of the inoculated plants grown in nonsolarized soil by 20–65% (Table 1) but did not affect plants in solarized soil. However, the population density of a streptomycin-resistant fluorescent pseudomonas (MS2) in the whole-root tissue and interior-root tissue of inoculated plants grown in solarized soil was significantly higher than in plants grown in nonsolarized soils (Fig. 1). Similar results were obtained when an additional streptomycin-resistant strain (MS10) was used (data not shown).

Vital staining of bacteria in inoculated roots from both nonsolarized and solarized soils showed intensive colonization of the root cortex surrounding the endodermis by bacteria (visible as purple-stained aggregates) (Fig. 2). Such aggregates also were observed in the vascular system but at a much lower frequency. The bacteria colonized lateral roots at an early stage of their generation; it appears that when the secondary roots emerge to the soil, their cortex tissue is already colonized with these bacteria. Bacterial aggregates were not visible in the roots of uninoculated plants.

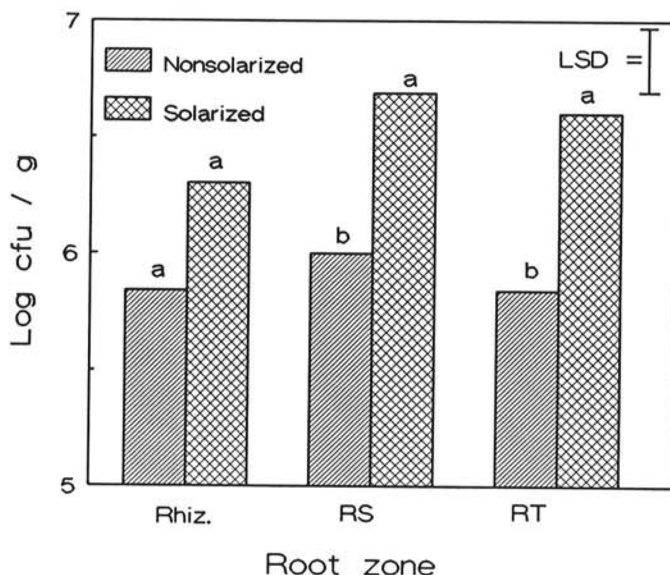

Fig. 1. Colonization of the rhizosphere (Rhiz.), whole-root tissue (RS), and interior-root tissue (RT) of tomato plants by a streptomycin-resistant strain of *Pseudomonas putida* (MS2) following root inoculation with the bacteria of plants in solarized and nonsolarized soils. Colonization of whole-root tissue was determined in washed and macerated roots. Colonization of interior-root tissue was determined in washed, disinfested, and macerated roots. A different letter within each root-zone fraction denotes significant differences between solarized and nonsolarized soils, according to Student's *t* test ($P \leq 0.05$); cfu = colony-forming units.

Interaction between fluorescent pseudomonads and the minor pathogen *Penicillium pinophilum*. Two isolates of *Penicillium pinophilum* reduced growth of tomato plants in nonsolarized soil by 44 and 42% at the high-inoculum density of 10^6 cfu per gram of soil (Table 2). The five tested strains of fluorescent pseudomonads increased growth of tomato seedlings in nonsolarized soil by 7–29% and nullified growth retardation by *Penicillium pinophilum. Pseudomonas fluorescens* and *P. putida* significantly reduced colonization by *Penicillium pinophilum* of roots of tomato plants grown in fungus-infested soil (Fig. 3). These bacteria reduced colonization of *Penicillium pinophilum* in the rhizosphere, whole-root tissue, and interior-root tissue by 62–75, 99, and 89–94%, respectively, as compared with plants that had not been inoculated with the bacteria. In contrast to fungal establishment, colonization of roots by fluorescent pseudomonads ranged from 10^7 to 10^8 cfu per gram of tissue in all treatments and was not significantly affected by the presence of *Penicillium pinophilum* (data not shown). Three strains each of *Pseudomonas putida, P. fluorescens,* and *P. alcaligenes* were tested on agar for antagonistic activity toward two isolates of *Penicillium pinophilum*. There was no evidence of antibiotic activity or lysis in any of the tested combinations.

Suppression of *Penicillium pinophilum* in solarized soil. *Penicillium pinophilum* suppressed growth of tomato plants when grown in nonsolarized soil (Fig. 4). This effect was even more pronounced in plants grown in methyl bromide-fumigated soil. The harmful effect of *Penicillium pinophilum* was less pronounced when plants were grown in infested, solarized soil. Dry weight of these plants at the highest inoculum concentration was 88–93% of that found in plants grown in noninfested, solarized soil. Dry weight of plants grown in the highest inoculum concentration in nonsolarized soils and methyl bromide-fumigated soils was 37–45 and 56%, respectively, of that found in plants grown in the respective noninfested soil. Statistical analysis to test a possible threshold of inoculum density demonstrated that significant reduction in dry weight of tomato plants occurred when inoculum density in soil was 10^4 cfu per gram or above in untreated or methyl bromide-fumigated soil.

Penicillium pinophilum also caused growth retardation of cotton plants in a separate experiment. Dry weight of cotton plants grown in *Penicillium pinophilum* infested soil (10^6 cfu per gram of soil) was significantly reduced, by 23–30% in nonsolarized soil and by 33–38% in methyl bromide-treated soil, as compared

TABLE 2. Effect of soil infestation with *Penicillium pinophilum* (ST11 and ST50) and of root inoculation with strains of fluorescent pseudomonads on dry weight of tomato plants in nonsolarized soil in pot experiments[x]

Bacteria[y] strain	Fungal isolate: None	ST11	ST50
None	570.0 A d[z]	320.0 B d	330.0 B c
RS7B	695.0 A b	586.3 C bc	639.7 B a
RS11M	610.0 A c	600.0 A c	570.0 A b
RS13M	641.7 A c	601.7 AB bc	593.3 B b
RB33M	643.3 A c	610.0 A b	611.0 A ab
RS34 M	736.7 A a	656.7 B a	645.0 B a

[x] Tomato seedlings were root-dipped in the bacterial suspension (10^8 colony-forming units per milliliter) and transplanted to noninfested soil or soil infested with conidia (10^6 per gram of soil) of the fungal isolate. Dry weight (milligrams per pot) was determined after 28 days of growth.
[y] RS7B is *Pseudomonas fluorescens*, RS11M and RS13M are *P. alcaligenes*, and RS33M and RS34M are *P. putida*.
[z] Experiment was conducted in a factorial design with six replicates (pots) per treatment and was repeated three times. Data shown represent the combined data from the three experiments. There was significant interaction between bacteria and fungi on dry weight. Thus, uppercase letters denote significant differences between fungal isolates within each bacterial strain, and lowercase letters denote significant differences between bacterial strains within each fungal isolate, according to Fisher's protected LSD test ($P \leq 0.05$).

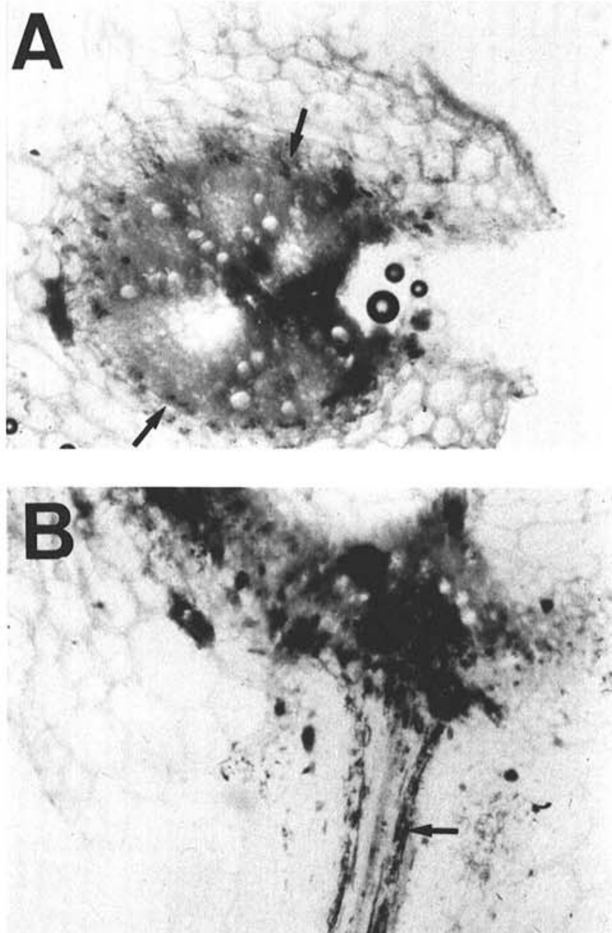

Fig. 2. Vital staining of bacteria in root tissue of tomato plants inoculated with *Pseudomonas putida* (RS34M), shown in cross section. Deeply stained aggregates of bacteria (arrows) are seen **A**, in the cortex surrounding the endodermis and **B**, in the longitudinal plane of the lateral roots.

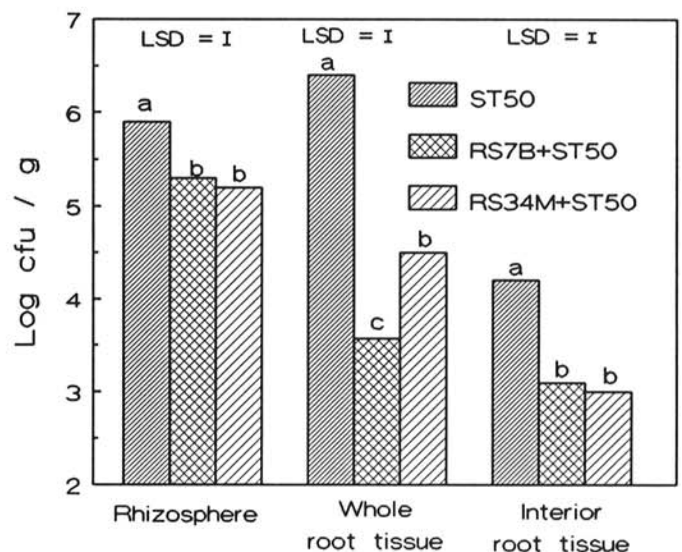

Fig. 3. Colonization by *Penicillium pinophilum* (ST50) of the rhizosphere, whole-root tissue, and interior-root tissue of tomato plants inoculated with *Pseudomonas fluorescens* (RS7B) or *P. putida* (RS34M). After inoculation, seedlings were planted in nonsolarized soil mixed with conidia of the fungus (10^6 per gram of soil). Experiment was conducted with six replicated pots per treatment and was repeated three times. Data shown represent combined data of the three experiments. Letters denote significant differences between treatments within each root-zone fraction, according to Fisher's protected LSD test ($P \leq 0.05$); cfu = colony-forming units.

to that found in plants grown in noninfested soil. As with tomato plants, no significant reduction in dry weight was observed in cotton plants grown in infested, solarized soil (10^6 cfu per gram of soil).

Roots of plants grown in either untreated or methyl bromide-fumigated soils infested with *Penicillium pinophilum* were colonized by the fungus in various levels, all of which were significantly higher at 10^6 conidia per gram of soil (Fig. 5) than were those measured in solarized soil. The intensified establishment of *Penicillium pinophilum* strongly suppressed colonization of roots by native populations of fluorescent pseudomonads in nonsolarized soil but not in solarized soil (Fig. 6). Bacterial colonization of roots in solarized soil was significantly higher than in the corresponding nonsolarized soil.

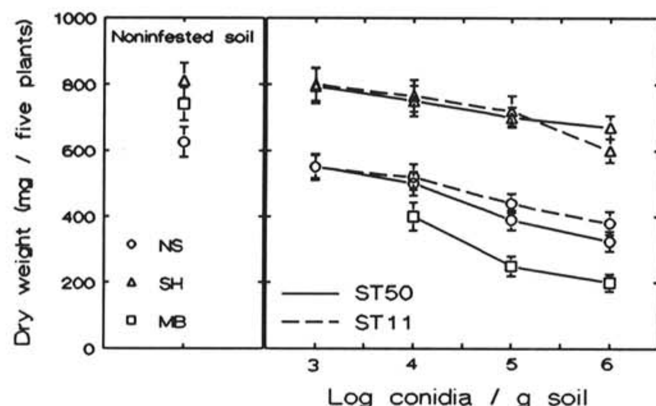

Fig. 4. Effect of *Penicillium pinophilum* (ST11 and ST50) on dry weight of tomato plants after 28 days of growth. Washed conidia of the fungus were mixed with nonsolarized (NS), solarized (SH), or methyl bromide-fumigated (MB) Rehovot soil. Experiment was conducted in a factorial design with six replicated pots per treatment and was repeated three times. Data represent combined data of the three trials. Significant interaction was evident between soil disinfestation and fungal infestation. Significant reduction in dry weight within each soil disinfestation was evident at an inoculum density of 10^4 and above, according to Fisher's protected LSD test ($P \leq 0.05$); cfu = colony-forming units. Vertical bars indicate standard error between the trials.

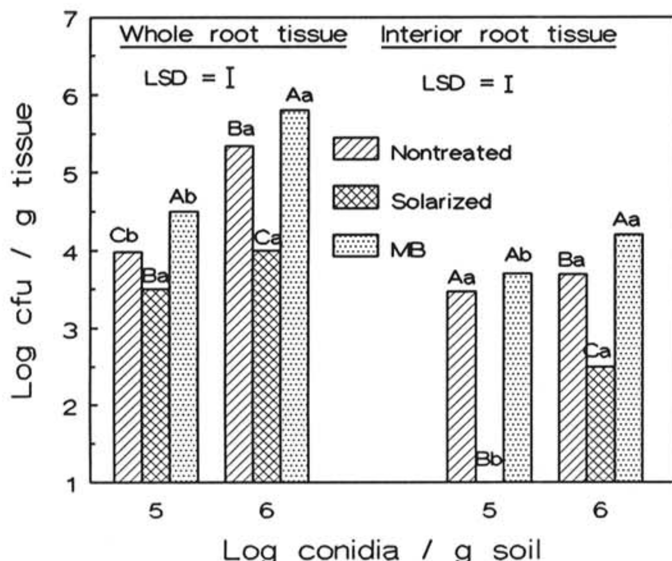

Fig. 5. Population densities of *Penicillium pinophilum* (ST50) in whole-root tissue and interior-root tissue of tomato plants grown in nontreated, solarized, or methyl bromide-fumigated (MB) Rehovot soil. Soil was infested with washed conidia of the fungus at two concentrations. Colonization of whole-root tissue was determined in washed and macerated roots. Colonization of interior-root tissue was determined in washed, disinfested, and macerated roots. Experiment was conducted in a factorial design with six replicated pots per treatment and was repeated three times. Data shown represent combined data of the three trials. There was significant interaction between the effect of the fungal concentrations and soil disinfestation. Thus, for each root, the uppercase letter denotes significant differences between soil disinfestation within each fungal concentration, and the lowercase letter denotes significant differences between fungal concentrations within each soil disinfestation, according to Fisher's protected LSD test ($P \leq 0.05$); cfu = colony-forming units.

Interaction between fluorescent pseudomonads and major pathogens. Disease incidence caused by Fusarium wilt in cotton and tomato and bean rot caused by *S. rolfsii* were effectively reduced by *Pseudomonas putida, P. fluorescens,* or *P. alcaligenes* (Table 3, Fig. 7). *Pseudomonas putida* and *P. fluorescens* reduced the incidence of Fusarium wilt of cotton by 90 and 84%, respectively (Fig. 7). Incidence of disease caused by *S. rolfsii* in bean was effectively reduced by the three species of bacteria, regardless of the origin of the pathogen sclerotia. Disease level was higher with sclerotia originating from diseased plants. The fluorescent pseudomonads also reduced the incidence of Fusarium wilt in tomato by 90–95% but did not affect Fusarium wilt in melons (data not shown).

The population density of *F. o. vasinfectum* in the tissues of infected cotton plants was reduced by 97–100% upon inoculation with *Pseudomonas putida* (Fig. 8). Similar results were obtained with *F. o. lycopersici* on tomato (data not shown). Population densities of native fluorescent pseudomonads in root tissue of bean were not affected by *S. rolfsii* infection in either solarized or nonsolarized soils but were reduced in fumigated soil (Table 3).

Suppression of major pathogens in solarized soil. Compared to nonsolarized soil, incidence of diseases caused by *F. o. vasinfectum* in cotton and *S. rolfsii* in bean was significantly reduced in solarized soil subsequently infested with the pathogen (Table 3, Fig. 7). In contrast, disease percentages in cotton and bean were 33 and 7–29% higher, respectively, when the plants

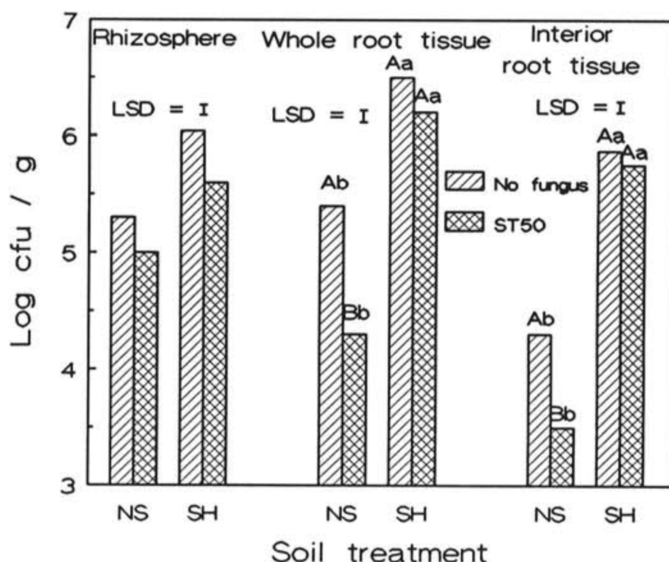

Fig. 6. Population densities of native fluorescent pseudomonads in rhizosphere, whole-root tissue, and interior-root tissue of tomato plants grown in nonsolarized (NS) or solarized (SH) soils infested with washed conidia of *Penicillium pinophilum* (ST50, 10^6 per gram of soil). Colonization of whole-root tissue was determined in washed and macerated roots. Colonization of interior-root tissue was determined in washed, disinfested, and macerated roots. Experiment was conducted in a factorial design with six replicated pots per treatment and was repeated three times. Data shown represent the combined data of the three trials. There was significant interaction between fungal inoculation and soil disinfestation on fluorescent pseudomonad density in the whole-root tissue and interior-root tissue but not in the rhizosphere. Thus, for the rhizosphere, only the differences between the main effects are given (i.e., population densities were significantly higher in solarized soil and with no fungus infestation compared to nonsolarized soil and fungal-infested soil, respectively). For each of the other two root parts, the uppercase letter denotes significant differences between soil disinfestation within each fungal infestation, and the lowercase letter denotes significant differences between fungal infestation within each soil disinfestation, according to Fisher's protected LSD test ($P \leq 0.05$); cfu = colony-forming units.

were grown in fumigated soil subsequently infested with the pathogen. Similarly, colonization of tissues of cotton plants by *F. o. vasinfectum* was suppressed in plants grown in solarized soil, especially in the upper part of the plants (Fig. 8). Population densities of fluorescent pseudomonads in root tissue of bean grown in solarized soil were higher than those in beans grown in the other two soils and were not affected by pathogen infection (Table 3). Population density of fluorescent pseudomonads in the tissues of plants inoculated with these bacteria was much higher than that in noninoculated plants. The germination percentage of *S. rolfsii* sclerotia adjacent to plants inoculated with fluorescent pseudomonads ranged from 90 to 100% and was similar to that of sclerotia adjacent to uninoculated plants.

DISCUSSION

In accordance with previous studies (8,17,23), fluorescent pseudomonads improved plant growth when added to nonsolarized soil (Table 1) but did not have the same effect in solarized soil. This might be due to the fact that roots of plants growing in solarized soil are intensively colonized by fluorescent pseudomonads, even without artificial inoculation with these bacteria (Table 3, [8]). Moreover, populations of microorganisms such as *Penicillium pinophilum* are drastically reduced in solarized soils (8). It is probable, therefore, that the beneficial contribution of supplementary, introduced pseudomonads toward further improvements in plant growth in solarized soil will be minimal. The early and intensive colonization of the interior parts of the roots by fluorescent pseudomonads gives these bacteria an advantage by facilitating their establishment in the roots. Apparently, fluorescent pseudomonads colonize new roots as they sprout by moving through the interior tissues, thus avoiding the hostile environment of the rhizosphere.

Fluorescent pseudomonads, it has been suggested, improve plant growth through the control of harmful microorganisms (23,24,28). Interaction between fluorescent pseudomonads and both minor pathogen *Penicillium pinophilum* and major pathogens was evident in this study. In both cases, the bacteria reduced the harmful effect of the fungi on tomato growth and suppressed the establishment of these fungi on root tissues. Similarly, the adverse effect of *Penicillium pinophilum* and major pathogens on plants was reduced in solarized soils and was associated with stimulated colonization of the rhizosphere and root tissues by native fluorescent pseudomonads. (8,27).

Suppression of *Penicillium pinophilum* and *F. o. vasinfectum* by fluorescent pseudomonads also was more pronounced in the roots than in the rhizosphere (Figs. 3 and 8). In addition to being competent rhizosphere colonizers (4,18,23,25), fluorescent pseudomonads seem to be highly competent colonizers of root tissue (8,29). This trait should be considered when screening for beneficial bacteria. Colonization of the rhizosphere and roots by fluorescent pseudomonads followed the same trends in both native and streptomycin-resistant strains. Possible mechanisms of the suppression of *Penicillium pinophilum* or its action by fluorescent pseudomonads include induced plant resistance and competition (antibiotic production and lysis excluded in this study). In the rhizosphere of plants growing in solarized soil, root exudates

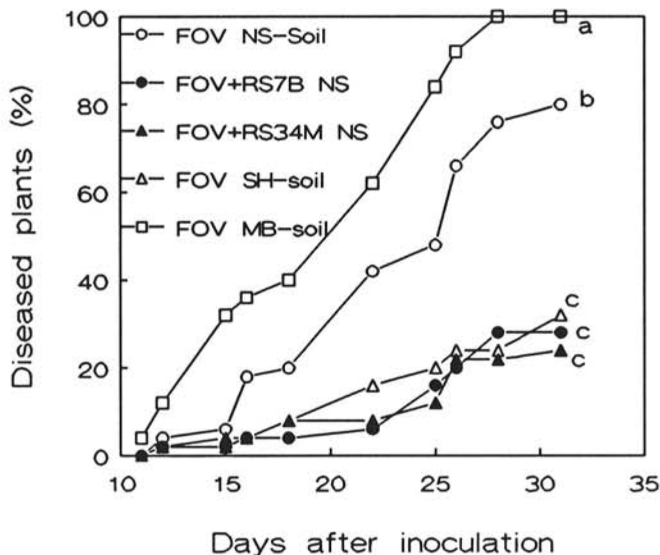

Fig. 7. Effect of *Pseudomonas fluorescens* (RS7B) and *P. putida* (RS34M) in nonsolarized (NS) soil and the effect of transplanting to solarized (SH) soil or methyl bromide-fumigated (MB) soil on the incidence of Fusarium wilt in cotton. Soils were infested with 10^5 conidia of the major pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) per gram of soil after disinfestation, with or without root dipping in bacterial suspension before planting. Different letters denote significant differences among treatments after 31 days, according to Fisher's protected LSD test ($P \leq 0.05$).

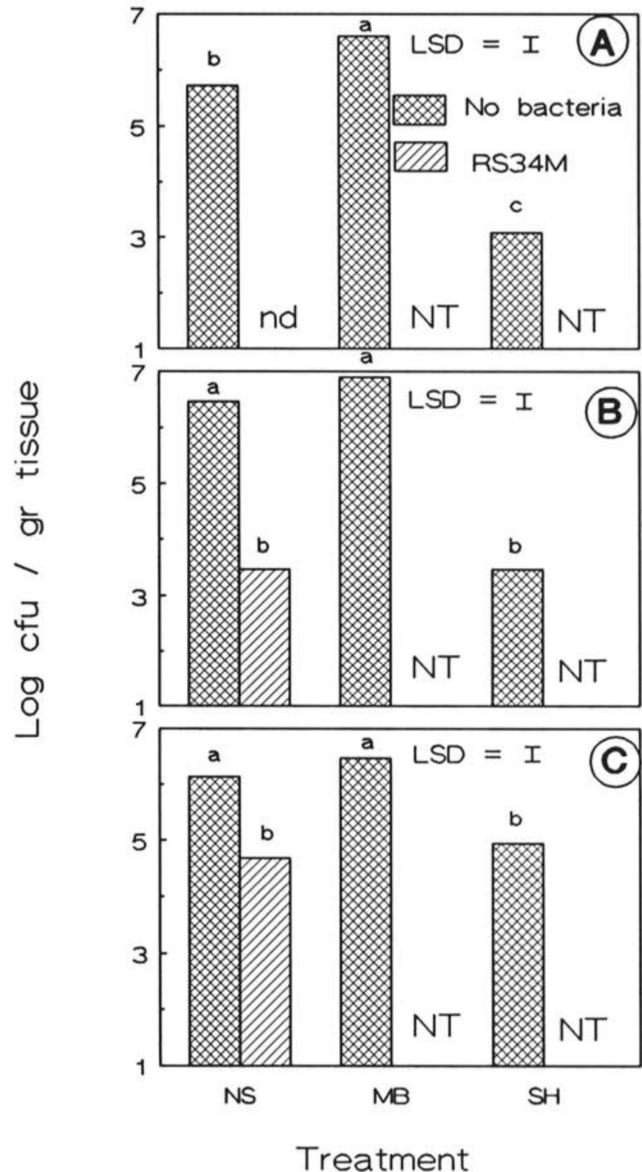

Fig. 8. Population density of *Fusarium oxysporum* f.sp. *vasinfectum* in the upper stem (**A**), hypocotyl (**B**), and root (**C**) tissues of cotton plants grown in nonsolarized (NS), methyl bromide-fumigated (MB), or solarized (SH) soils or of cotton plants after dipping roots in a *Pseudomonas putida* (RS34M) suspension prior to planting in nonsolarized soil. Soils were mixed with washed conidia of the pathogen (2×10^5 per gram of soil). Experiment design utilized six replicated pots per treatment and was repeated three times. Data shown represent the combined data from the three trials. Different letters within each plant part denote significant differences between treatments after 31 days of growth, according to Fisher's protected LSD test ($P \leq 0.05$); cfu = colony-forming units; nd = not detectable; NT = not tested.

differ in quality and composition from those in nonsolarized soil (9); this may affect competition.

Penicillium pinophilum was characterized as a minor pathogen because, in this study (Fig. 4) and others (8,9), it significantly reduced dry weight of tomato and cotton plants grown in soil it infested. Unlike major pathogens, minor pathogens are difficult to describe. They do not cause typical symptoms seen with many destructive diseases; instead, they cause growth retardation and stunting. Their expression depends on environmental factors, inoculum density, and interaction with other microorganisms (22,30). It was shown here that growth retardation caused by *Penicillium pinophilum* in untreated and methyl bromide-fumigated soils depends on an inoculum-density threshold of 10^4 propagules per gram of soil (Fig. 4). The natural occurrence of this fungus in soils, as found in an earlier report (8), is 10^3–10^4 propagules per gram of soil, which is at or below the inoculum-density threshold. This may explain the fact that plants growing in a field under normal conditions suffer minimal growth suppression and do not show strong symptoms unless they are compared to plants grown in disinfested soils. Furthermore, inoculum densities exceeding 10^4 propagules per gram of soil were recorded in fields where cotton and tomato crops were continuously grown for several years and where the effects of disease on yield were well-defined (A. Gamliel and J. Katan, *unpublished data*). In greenhouse experiments, as described in this study, we used high-inoculum densities to amplify the effect of *Penicillium pinophilum* on tomato transplants within a short time span, to determine its potential as a minor pathogen. To test the effect of low-inoculum densities on growth retardation, a longer period of growth is required, during which a broad spectrum of biotic and abiotic factors can affect plant response.

The establishment of native as well as introduced *Penicillium pinophilum* in the rhizosphere and roots of plants in solarized soil is suppressed, reducing its adverse effect on plant growth. Obviously, the induced-suppressiveness phenomenon against major pathogens in solarized soil, as shown here and elsewhere (10), also is manifested against an indigenous minor pathogen (8) that affects the growth of both tomato and cotton plants. This reflects the fact that major as well as minor pathogens are soilborne organisms colonizing the rhizosphere and reproducing within plant tissues. Also, both groups are suppressed by fluorescent pseudomonads, which suggests that these bacteria may be involved in the induced-suppressiveness phenomenon. In contrast to the suppressive nature of solarized soil, soil fumigation did not induce suppressiveness nor did it increase populations of fluorescent pseudomonads. Moreover, in certain cases it even enhanced the activities and the detrimental effect of both *Penicillium pinophilum* and pathogenic fungi introduced into the soil after fumigation. A biological vacuum seems to be created following fumigation, facilitating the establishment of pathogenic fungi (5,10,11,20).

Fluorescent pseudomonads effectively controlled *S. rolfsii, F. o. vasinfectum* (Table 3, Fig. 7), *F. o. lycopersici*, and *F. o. radicis-lycopersici* (A. Gamliel and J. Katan, *unpublished data*) but not *F. o. melonis*. This suggests that the interaction between these bacteria and major pathogens have some pattern of specificity, rather than a simple competition between two microorganisms on the same root niche. The biocontrol capacity of fluorescent pseudomonads is well documented (4,17,23,24). It is probable that this capacity, coupled with the capacity to rapidly colonize the rhizosphere and root tissues in a variety of solarized soils (8), contributes to the development of induced suppressiveness against major and minor pathogens (Tables 2 and 3, Fig. 7) (6,10,11,14), as well as other mechanisms (e.g., suppression of chlamydospore formation [10]), in solarized soils. Fluorescent pseudomonads reduced the incidence of disease caused by *S. rolfsii* in bean but did not affect the germination capacity of the sclerotia, indicating that inoculum potential rather than inoculum density is affected.

Penicillium pinophilum and *S. rolfsii* did not affect root colonization by fluorescent pseudomonads added to soil, nor did *Penicillium pinophilum* suppress colonization in the rhizosphere and roots by native fluorescent pseudomonads in solarized soil. However, *Penicillium pinophilum* did suppress colonization of roots by native fluorescent pseudomonads in nonsolarized soil (Fig. 6). Such a suppression of potentially beneficial microorganisms also could contribute to the retardation of plant growth by the minor pathogen.

The ability of fluorescent pseudomonads to improve plant growth in a given soil depends on the genetic structure of their

TABLE 3. Control of *Sclerotium rolfsii* (SR) of bean by fluorescent pseudomonads (FP) in untreated or treated soils and colonization of root-neck tissue by these bacteria

Soil disinfestation	Bacterial treatment[b]	SR[c]	Disease (%) Culture[d]	Disease (%) Plant	FP density[a] (log cfu/g)
Nonsolarized	None	−	0	0	4.78 A c
	None	+	72 A b[e]	93 A a	4.39 A b
	Pseudomonas putida (RS34M)	+	25 B a	37 B a	NT
	P. fluorescens (RS7B)	+	16 B a	29 B a	NT
	P. alcaligenes (RS32M)	+	36 B	45 B	NT
Solarized	None	−	0	0	6.80 A a
	None	+	20 A c	32 A b	6.95 A a
	P. putida (RS34M)	+	10 A a	15 B a	NT
	P. fluorescens (RS7B)	+	12 A a	10 B b	NT
Methyl bromide fumigation	None	−	0	0	5.7 A b
	None	+	93 A a	100 A a	4.7 B b
	P. putida (RS34M)	+	10 B a	22 B b	NT
	P. fluorescens (RS7B)	+	12 B a	18 B ab	NT

[a] Population density of fluorescent pseudomonads determined in surface-disinfected tissues of the lower stem after 21 days; cfu = colony-forming units; NT = not tested.

[b] Bean seedlings (2 days after germination) were soaked in a bacterial suspension (10^8 cfu per milliliter) for 10 min and transplanted to nonsolarized, solarized, or methyl bromide-fumigated soils.

[c] Sclerotia were placed in soil near each seedling. Disease was determined after 21 days; + = inoculated; − = uninoculated.

[d] Sclerotia were collected from either a culture or from the soil surface around diseased bean plants. Sclerotia were placed in soil adjacent to germinating bean seeds.

[e] Experiment was conducted in a factorial design with six replicates (pots) per treatment. The experiment was repeated three times, and the data shown represent the combined data of the three experiments. There was significant interaction between bacteria and soil disinfestation and disease incidence. In each disease column, uppercase letters denote significant differences between bacterial strains within the same soil, and lowercase letters denote significant differences between soil disinfestation within each bacterial strain. For FP-density column, uppercase letters denote significant differences between SR inoculation within the same soil, and lowercase letters denote significant differences between soil disinfestation within each SR inoculation, according to Fisher's protected LSD test ($P \leq 0.05$).

populations in that soil, on the biotic and abiotic environmental conditions prevailing in the root zone, and on the agricultural practices employed, (e.g., soil disinfestation). Plant health depends on, among other factors, the balance between the beneficial and harmful biotic agents present. The relationship between improved plant growth and biocontrol capacities deserves further study, because results may facilitate the screening of beneficial microorganisms.

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