Growth Promotion of Apple Seedlings and Rootstocks by Specific Strains of Bacteria

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We thank B. H. Katz for helpful advice and assistance and the Office of Indian Education, U.S. Department of Education, for research funds.

Accepted for publication 25 June 1987 (submitted for electronic processing).

ABSTRACT

Caesar, A. J., and Burr, T. J. 1987. Growth promotion of apple seedlings and rootstocks by specific strains of bacteria. Phytopathology 77:1583-1588.

Treatment of apple seedlings and rootstocks with strains of bacteria isolated from plant roots resulted in significant growth increases—up to 65% in seedlings and up to 179% in rootstocks. Strains were tested for growth-promoting activity on apple seedlings and rootstocks M.7 and M.26 in greenhouse and field trials. Strains were also tested for broad-spectrum in vitro antibiosis against six to 12 apple root-infecting fungi, including *Fusarium oxysporum, Alternaria alternata, Pythium* spp., and *Rhizoctonia solani*. Nineteen strains that were isolated from roots and showed in vitro antibiosis against other microorganisms on dilution plates were also tested for growth promotion. Growth responses were correlated with the detection of fewer root-associated fungi on rootstocks and with a lower frequency of root colonization by *Cylindrocarpon destructans*. Rootstocks treated with the plant growth-promoting rhizobacteria (PGPR) had up to 102% more active lateral root nodes. The most effective PGPR strains were fluorescent *Pseudomonas* spp. and enteric bacteria.

The application of bacteria to seed and vegetative propagation material to increase plant growth has been studied in recent years on several crops (1,8,27). Treatment of potato (8,21), sugar beets (31), wheat (6), and other crops (10,11,18,34) with plant growth-promoting rhizobacteria (PGPR) often results in significant growth and yield increases. Generally, increases were obtained when high levels of bacteria were applied to seed or seed pieces that were planted in moist, well-drained, fertile soils. Many PGPR have been identified as fluorescent *Pseudomonas* spp. It has been suggested that growth increases are due in part to an alteration of the rhizosphere microflora and a reduction of various fungal species that infect the root cortex (29,34).

There are few reports on the effects of PGPR on perennial crops. Previous research has described growth increases of apple seedlings (33) and apple rootstocks (11) grown in replant soils after treatments with strains of *Pseudomonas* spp. Treatment of bare rootstocks of almond with a strain of *Agrobacterium rhizogenes* was reported to increase early growth of trees (28). Biological control of crown rot of apple, caused by *Phytophthora cactorum*, using enteric bacteria (32) or *Pseudomonas* spp. (15) has also been reported.

The purpose of this research was to identify the effects that strains of rhizosphere bacteria isolated from different plants have on the growth of apple. Comparative studies related in vitro antibiosis and the effects of strains on the rhizosphere microflora to the growth of apple seedlings and rootstocks.

MATERIALS AND METHODS

Selection of candidate bacterial strains. Candidate PGPR strains were isolated from roots of apple and rosaceous wild hosts, including wild grape (*Vitis* spp.), *Potentilla, Prunus, Fragaria, Rosa, Pyrus, Rubus, Malus, Crataegus*, and *Spiraea* spp., and from a number of common weed species. Freshly dug roots of host plants were shaken to remove loosely adhering soil, placed in 0.1 M magnesium sulfate or 0.1 M phosphate buffer (pH 7) with 0.1% (w/v) proteose peptone, and shaken for 1 min on a vortex mixer. Dilutions were plated on King's medium B agar (KB) (17), nutrient agar (Difco Laboratories, Detroit, MI), and eosin methylene blue agar (BBL Microbiology Systems, Cockeysville, MD). Predominant colony types were selected from isolation plates, as were strains that showed antibiosis against microflora growing on the same plate. Isolation and purification techniques were similar to those of earlier workers (8,18,29). Strains were stored in small test tubes containing sterile water or 0.1% proteose peptone at 4 C.

Greenhouse and in vitro screening of candidate strains. A total of 226 strains were tested for growth-promoting activity on apple seedlings in the greenhouse. The strains were spread onto plates of KB and incubated at 25 C for 24–96 hr. The bacteria were then scraped off and suspended in 1% (w/v) methylcellulose (31). Stratified and germinated seed of the apple cultivar McIntosh were soaked in the bacterial suspension for 30 min to 1 hr, then removed and coated with sterile talc. The number of bacteria per treated seed was approximately 10^8 colony-forming units (cfu), determined by shaking 10 individual seeds in 9.0 ml of phosphate buffer and plating dilutions on KB.

At least 25 seeds were planted for each treatment in a 33% (v/v) field soil/sand mixture in 7.5-cm diameter, 7.5 cm deep plastic pots or 4-cm diameter, 15 cm deep plastic cones (R. Leach Conetainer Nursery, Canby, OR). The field soil was a clay loam (pH 5.9) from a commercial nursery that had been planted to several successive crops of apple rootstocks. Pots were watered from beneath as needed to keep soil moist without saturation; cones were watered from above. Most screening was done under ambient light during the spring and fall, with greenhouse temperatures ranging from 20 to 25 C. Seedlings were not fertilized during the experiment. After 6 wk, seedlings were harvested and fresh weights were recorded. Strains that resulted in significantly larger seedlings were saved for further testing.

A total of 258 strains from roots were tested for in vitro antibiosis against six to 12 apple root-infecting fungi. These included most but not all of the same strains tested for growth promotion of apple seedlings. The apple root-infecting fungi included two isolates of *Fusarium oxysporum* and an unidentified *Fusarium* sp., three of *Pythium* spp., and one of *Alternaria alternata*. Some strains were further tested against two isolates of *Phytophthora cactorum* and one of *P. megasperma* and a multinucleate isolate of *Rhizoctonia solani*. Strains were streaked, three per plate, near the outer edge of 15 × 100 mm petri dishes containing Difco potato-dextrose agar or cornmeal agar and incubated at 25 C for 36–48 hr. A 3-mm-diameter mycelial plug of the test fungus cut from the margin of an actively growing culture was transferred to the center of each plate. Plates were incubated at 25 C and assessed for antagonism after 36 hr for pythiaceous fungi, 48 hr for *Rhizoctonia*, and 72–96 hr for *Fusarium* and *Alternaria*. Positive antibiosis was recorded if the inhibition zone was at least 0.5 cm wide at the time of initial measurement and remained so for at least 36 hr.

Testing of bacteria on rootstocks. Strains that stimulated significant growth increases of seedlings and showed broad-spectrum in vitro antibiosis were tested in the greenhouse and field on the apple rootstocks M.7 and M.26. Initially, all roots were pruned from rootstocks and rootstocks were soaked in 1.05% (v/v) sodium hypochlorite for 30 min to reduce the surface microflora. Rootstocks were then rinsed in running tap water and individually weighed. Inoculum was produced by growing bacteria at 20–25 C on 150 × 15 cm petri plates of KB for 6–7 days. Growth of inoculum for this period is reported to improve subsequent rhizosphere colonization and survival (6,26). Methylcellulose (1%) suspensions of cultures were added to a 5% (w/v) xanthan gum gel. Xanthan gum, an effective carrier for PGPR (19), was shown to be nontoxic to several PGPR strains (9) and alone had no significant effect on rootstock growth in two different soils over 9 wk in greenhouse studies (Caesar, *unpublished*). This formulation was quite viscous, and when rootstocks were dipped in a bacterial suspension of 10^9 cfu ml^{-1} and coated with sterile talc, a formulation about 0.5–1.0 mm thick adhered to them. This resulted in populations of 10^7–10^8 cfu per cm^2 of the rooting surface of rootstocks (9). Rootstocks were treated with bacterial formulations and planted in the field soil/sand mixture in waxed, 0.946- or 1.89-L cardboard milk containers in the greenhouse.

A mixture of three strains (Hi, Ro, and WG18NF) was applied to determine if this combination caused additional growth over single-strain inoculations. The root-colonizing ability of strains (9) was tested simultaneously using rifampicin-resistant (marked) substrains. The marked mixture, RCombo, comprised spontaneous mutants of each strain selected from KB agar plates amended with rifampicin at a concentration of 100 μg per milliliter.

Field plot studies were conducted in commercial apple nurseries in central New York using positive strains from previous screenings. Two sites, Stanley 1984 and Wolcott 1985, had been planted continuously to apple rootstocks for several years, unlike the rest of the sites. Soil types varied with location: Stanley 1984 was a Lima silt loam, pH 5.9; Stanley 1985 was a Honeoye fine sandy loam, pH 6.2; Wolcott was a Collamer silt loam, pH 5.7; and Fruit Testing 1986 was a Honeoye silt loam, pH 6.4. Field plots were nonirrigated except the 1985 Stanley and Wolcott plots, which were sprinkler-irrigated every 1–2 wk. Rootstocks were planted in a randomized complete block design in early to mid-May and harvested in late October the same year. Plots were fertilized with rates equivalent to 54 kg/ha of 1:1:1 ($N:P_2O_5:K_2O$) 1 wk before planting. Growth increases in field trials were determined by measuring weight gains in relation to initial rootstock weight because the initial weight affects growth and final weight. The normality of proportional weight gain data sets was established using the Kolmogorov test (for data sets larger than 51) contained in the univariate procedure of SAS (Statistical Analysis Systems, release 4.08, SAS Institute Inc., Cary, NC).

Effect of applied bacteria on rhizosphere bacteria. The total aerobic bacteria and gram-positive bacteria associated with PGPR-treated roots was determined. Since accurate assessment of bacterial populations requires that counts be taken from entire root systems (23), roots were collected from each treatment. Roots from each rootstock were placed in sterile 0.1% (w/v) proteose peptone in 50-ml Erlenmeyer flasks with 25 ml of 0.1% peptone and phosphate buffer (pH 7), stirred for 1 min on a vortex mixer, allowed to stand for 5 min, and stirred again for 30 sec. Then, 0.1-ml aliquots of serial dilutions were plated in triplicate on tryptic soy agar (25) or on a medium described as being selective for gram-positive bacteria (PDC-NA) (20). In subsequent experiments, PDC-NA was not used because high populations of gram-negative bacteria were detected on dilution plates of this medium. Roots from five rootstocks per treatment (M.7 and M.26) were evaluated separately from greenhouse experiments. Populations were expressed as cfu/g fresh weight of root.

Effect of bacteria on colonization of roots by pathogenic fungi. The effect of bacterial treatments on the fungi associated with roots of rootstocks was assessed in the greenhouse trials in the summer of 1984 and in all field trials in 1984 and 1985. Roots were washed in 1% (w/v) sodium hexametaphosphate/0.1% (v/v) Tergitol and plated on a cellophane extract agar (CEA) (34). Entire root systems from each plant within treatments (25–30 plants) were cut into 3- to 5-cm long pieces, added to 600–1,000 ml of the wash solution, and shaken on a wrist-action shaker for about 3 hr. Roots were then rinsed several times with tap water, shaken again for 1 hr, rinsed, and plated on CEA. Roots about 1–1.5 mm in diameter and without apparent infections were selected for plating to assess fungal populations. Roots were arranged parallel to each other and pressed with sterile forceps into the medium along 10-cm lines drawn on the bottom of 150 × 15 cm petri plates of CEA (Fig. 1). Counts were made 5–7 days after plating, and individual colonies were identified from CEA plates after 7–14 days or by transfer to PDA, CMA, or PARP (16) medium. Root infections by pythiaceous fungi and *Rhizoctonia* spp. were counted using PARP medium and water agar with 50 μg ml^{-1} of streptomycin and 50 μg ml^{-1} of chloramphenicol, respectively. In 1985, Tergitol NP-10 (0.1%) was added to both media to restrict the growth rate of fungal colonies and thus facilitate counting of individual colonies (2).

Effect of bacteria on lateral root emergence on rootstocks. The number of nodes along the stalk of the rootstock from which lateral roots emerged was counted for each treatment. The initiation and growth of at least one lateral root from a single node resulted in that node being designated as an active node. The number of active nodes was counted for all trees in all treatments of M.26 rootstocks and in one plot of M.7 in 1984. In 1985, active nodes were counted for both rootstocks in all treatments in field plots.

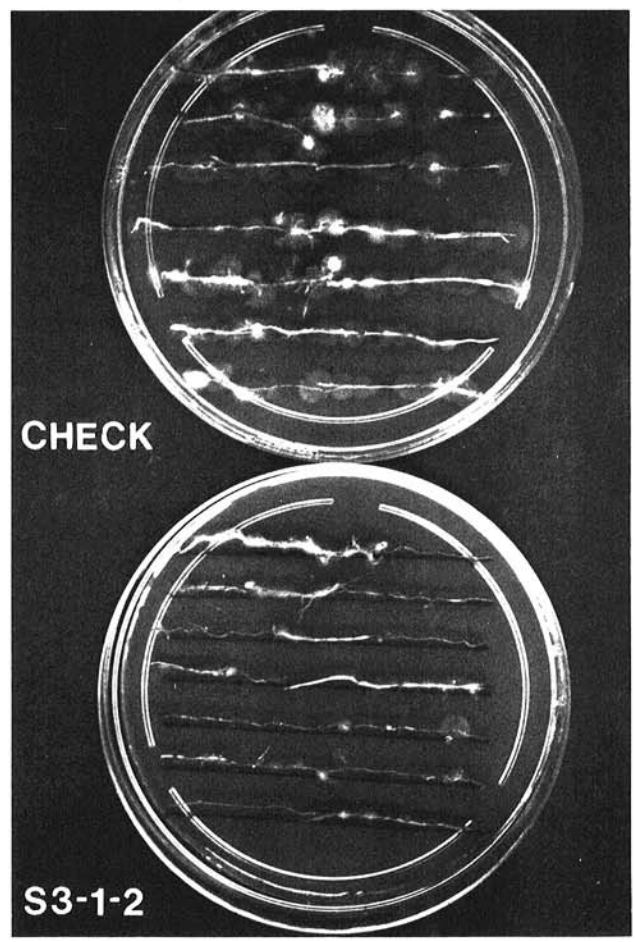

Fig. 1. Fungal colonies emerging from apple root pieces washed in 1% sodium hexametaphosphate and Tergitol and plated on cellophane extract agar. Roots treated with plant growth-promoting rhizobacteria (strain S3-1-2) have fewer fungi than check roots.

RESULTS

Effects on seedling growth and in vitro antibiosis. Thirteen strains resulted in significantly greater (23–47%) fresh weight of apple seedlings (Table 1). The majority of the strains either did not produce significant increases or had erratic results.

Of 258 strains, including those tested on apple seedlings, 12 showed in vitro antibiosis against all six test fungi. Four strains, Hi, Ro, S3-1-2, and S3-2-RP2, were also inhibitory to at least two of three strains of *R. solani* and the two *Phytophthora* spp.

Greenhouse testing of bacteria on rootstocks. Rootstock M.26 had a significantly ($P = 0.05$) greater weight, ranging from 38 to 60% when treated with strain WG18NF, Ro, or Hi. Four strains applied to rootstock M.7 gave significant growth increases up to 121%. The two most active strains were Hi and Ro, with respective weight increases of 60 and 45% on M.26 and 89 and 121% on M.7 (Table 2). In an additional test, Ro caused a 33% weight increase on M.7. The rifampicin-resistant mixture of strains Hi, Ro, and WG18NF (RCombo) gave a yield increase of 33% on rootstock M.26 and 19% on M.7. Treatments with significant weight gains had observable increases in root density, length, and top growth. The unmarked combination of strains (Combo) did not increase growth of either rootstock.

Effects of PGPR on rhizosphere bacteria. Strains that promoted growth of rootstocks did not affect the number of total bacteria or gram-positive bacteria on roots. Roots of PGPR-treated rootstocks had generally, but not significantly, higher cfu/g^{-1} of root of total bacteria. Logarithmic transformation of bacterial populations were also compared, with similar results.

Bacterial treatments consistently limited the number of root-associated fungi, including a few instances where significant growth promotion did not occur (Table 2). Significant weight gains of rootstocks were usually associated with populations of root-associated fungi that were about 15% less than nonbacterial-treated checks. An exception was strain S3-1-2, which resulted in only a 3% reduction but a yield increase of 74%.

TABLE 1. Growth increases of apple seedlings by strains of plant growth-promoting rhizobacteria in the greenhouse[a]

Bacterial strain	Significant[b] growth increases/number of tests	Mean growth increase (%)
WG18NF	3/3	40.3
PotNF	2/4	39.0
WG6-2	2/3	30.0
Ith2-2	2/5	29.5
WG15NF	1/2	47.0
Ra2-2	1/4	44.0
8205	1/2	41.0
A3-1	1/5	38.0
Ro	1/1	37.0
CG407	1/1	32.0
WG9	1/1	32.0
WG8	1/2	29.5
P3	1/2	23.0

[a] At least 25 germinated McIntosh seeds were inoculated with each strain. Only strains resulting in significant growth increases are listed.
[b] According to Waller and Duncan's exact Bayesian k-ratio LSD rule ($P = 0.05$).

TABLE 2. Effects of plant growth-promoting rhizobacteria on growth and root-associated fungi of apple rootstocks in the greenhouse[a]

Date Rootstock	Bacterial strain	Mean weight gain (g)	Weight increase (%)	Reduction in root-associated fungi[b] (%)
Spring 1984				
M.26	Hi	6.4*	60	15
	Ro	5.8*	45	45*
	WG18NF	5.5*	38	55*
	Ra2-2	4.8	20	16
	S3-1-2	4.5	12	30*
	Check	4.0	...	...
M.7	Ro	4.2*	121	30*
	Hi	3.6*	89	21*
	S3-1-2	3.3*	74	3
	WG18NF	3.0*	57	20*
	Check	1.9	...	...
Summer 1984				
M.26	RCombo	6.4*	36	20*
	Ro	4.9	4	12*
	Combo	4.8	2	10
	Check	4.7	...	...
M.7	Ro	7.7*	33	27*
	RCombo	6.9	19	12*
	Combo	5.9	2	15*
	Check	5.8	...	...

[a] Twenty-five rootstocks of each type were treated with each strain and planted in a 1:3 mixture of field soil to sand. * = Significant at $P = 0.05$ compared with check according to Waller and Duncan's exact Bayesian k-ratio LSD rule.
[b] Number of discrete colonies per centimeter of root length.

TABLE 3. Effects of plant growth-promoting rhizobacteria on growth and root-associated fungi of apple rootstocks in the field[a]

Location and date Rootstock	Bacterial strain	Mean weight gain (g)	Proportional weight gain[b]	Weight increase (%)	Reduction in root-associated fungi (%)
Stanley 1984					
M.26	Hi	22.9	1.04*	65.0	0.0
	WG18NF	18.7	0.83*	36.3	14.0*
	S3-1-2	17.5	0.82*	28.0	28.0
	Check	13.8	0.58	...	...
M.7	Hi	15.9	0.73*	49.1	15.0
	S3-1-2	15.6	0.71*	46.0	37.0*
	WG18NF	13.2	0.58	23.0	3.0
	Check	10.8	0.48	...	...
	WG-1	14.1*	NA	54.0	0.0
	Ro	9.5	NA	5.0	0.0
	Check	9.0	NA	...	...
	P7NF	8.3	NA	−7.7	0.0
Stanley 1985					
M.7	Hi	64.9	1.8*	104.0	0.0
	Ro	49.7	1.4	56.0	40.2*
	S3-1-2	41.9	1.1	32.0	14.5*
	Check	31.8	0.8	...	...
M.26	Ro	72.4	1.8*	179.0	17.9*
	S3-1-2	71.8	1.7*	176.0	28.5*
	Hi	48.4	1.5*	86.2	27.4*
	Check	26.0	0.7	...	...
Fruit Testing 1985					
M.7	Ro	49.1	1.5*	19.7	40.6*
	Hi	49.0	1.3	19.5	1.7
	Check	41.0	1.1	...	...
	S3-1-2	32.6	−0.9*	−22.0	19.2*
M.26	Ro	93.9	2.4	16.2	20.1*
	S3-1-2	82.2	2.3	1.8	15.6*
	Check	80.8	2.3	...	...
	Hi	52.1	1.5	−35.0	14.3*
Wolcott 1985					
M.7	S3-1-2	6.1	0.18	32.0	15.1
	Ro	4.8	0.13	2.0	6.9
	Check	4.7	0.12	...	...
	Hi	4.2	0.12	−10.0	12.7
M.26	Ro	11.8	0.24*	59.5	15.1
	Hi	9.1	0.20	23.0	6.9
	Check	7.4	0.15	...	...
	S3-1-2	4.4	−0.10*	−40.0	4.0

[a] In early November of years planted, 25–30 trees were harvested per each of five replications per field trial. * = Significant at $P = 0.05$ compared with check according to Waller and Duncan's exact Bayesian k-ratio LSD rule. Normality of proportional weight gains determined with Kolmogorov test (for data sets larger than 51).
[b] Proportion of original rootstock weights before treatment. NA = not assessed.

Field trials of PGPR on rootstocks. Three strains repeatedly caused greater growth of rootstocks M.7 and M.26 in field trials over a 2-yr period (Table 3). In 1984, significant yield increases were obtained with strain Hi on rootstocks M.26 and M.7. S3-1-2 significantly increased the growth of both rootstocks, and WG18NF caused a significant growth increase on M.26. Strain WG-1 gave a significant weight gain on rootstock M.7 in one experiment.

In 1985, strain Ro caused significant growth increases at all sites and on both rootstocks. At the Stanley site, strain Hi caused significant growth increases on both rootstocks and S3-1-2 caused significant increases on M.26.

Differences in trunk growth and root development between treatments were noticeable (Fig. 2), but shoot differences were not always apparent.

Effects on root-associated fungi. Significant growth increases in field trials were frequently associated with lower recoveries of root-associated fungi ranging from 14 to 40.6% (Table 3). Significant weight gains were not correlated with reductions with two treatments in one experiment, however. Counts of *Rhizoctonia* spp. and pythiaceous fungi were less than one colony per 10 cm of root for all treatments, including the check.

Treatment with PGPR consistently caused a reduction of *Cylindrocarpon destructans* as a percentage of total fungal species isolated from roots (Table 4). Reductions ranged from 6.5 to 24.1%. The identification of *C. destructans* was based on the morphology of colonies and chlamydospores, microconidia, and macroconidia on PDA (3) and CEA. Greenhouse studies, however, did not identify significant reductions of any single fungal species, although total fungi were reduced on roots of bacteria-treated rootstocks (Table 2).

Effect of bacteria on lateral root emergence. Although growth increases of M.26 from the Stanley 1984 trial were not associated with lower populations of root-associated fungi, rootstocks showed a greater number of active nodes (Table 4). Strains Hi and S3-1-2 caused significantly greater weight gains and numbers of active nodes on M.26, whereas strain WG-1 produced the same effects on M.7. In 1985 field trials, significant weight gains were associated with significantly greater numbers of active nodes in two of three field plots. Strains Hi and Ro consistently stimulated a greater number of active nodes, even when weight gains were not significant.

TABLE 4. Effects of plant growth-promoting rhizobacteria on lateral root formation and root colonization by *Cylindrocarpon*

Location and date Bacterial strain	Mean number of active nodes/root[a]		*Cylindrocarpon* (%)[b]	
	M.7 rootstock	M.26 rootstock	M.7 rootstock	M.26 rootstock
Stanley 1984				
Hi (M.7, M.26[c])	NT	11.7 x	15.3	53.3
WG18NF (M.7)	NT	11.7 x	11.6	30.7
S3-1-2 (M.7, M.26)	NT	10.3 xy	37.6	52.9
Check	NT	8.1 yz	31.1	41.9
WG-1 (M.7)	13.8 x	NT	42.2	NT
Ro	11.6 y	NT	70.0	NT
Check	9.8 y	NT	76.3	NT
P7NF	9.4 yz	NT	61.1	NT
Stanley 1985				
Hi (M.7, M.26)	10.2 x	9.5 y	5.4	8.1
Ro (M.26)	10.7 x	12.7 x	10.5	11.4
S3-1-2 (M.26)	11.1 x	11.1 xy	17.2	12.7
Check	6.2 y	6.3 z	29.0	19.8
Fruit Testing 1985				
Ro (M.7)	9.1 x	7.8 x	38.2	17.2
Hi	8.8 x	8.2 x	21.8	3.8
Check	8.0 x	7.3 x	50.4	28.8
S3-1-2	7.6 x	7.2 x	33.3	21.2
Wolcott 1985				
S3-1-2	NT	NT	77.7	75.2
Ro	NT	NT	70.2	80.8
Check	NT	NT	86.8	84.0
Hi	NT	NT	70.2	90.0

[a] Active nodes were determined by counting number of sites along stalk of rootstock from which lateral roots originated from preexisting nodes. Mean numbers of active nodes followed by same letter are not significant at $P = 0.05$ according to Waller and Duncan's exact Bayesian k-ratio LSD rule. Evaluations were made at harvest (early November). NT = not tested.

[b] Populations of root fungi were evaluated using cellophane extract agar (34). All colonies were identified as *C. destructans*. NT = not tested.

[c] Rootstocks on which growth increases were significant.

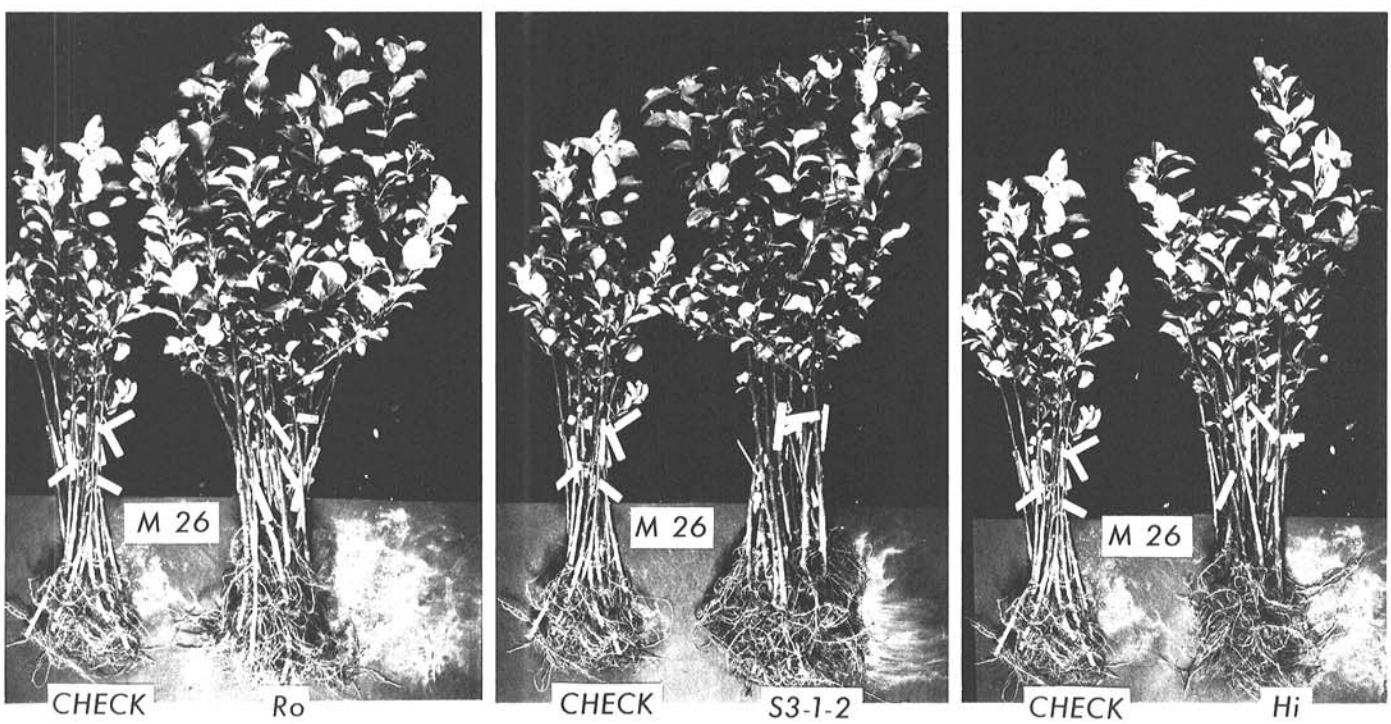

Fig. 2. Comparisons of apple rootstock M.26 treated with three strains of plant growth-promoting rhizobacteria or untreated.

DISCUSSION

Increased growth of apple rootstocks in nurseries by PGPR is particularly significant because maximum tree growth during the first year is essential. Growth responses were achieved in soils that had been previously planted to apple (replant) and in "new" sites. Increases up to 179% were obtained. Tree growth in replant soils was still less than in new soils, however, indicating that PGPR strains may not affect or adequately control factors associated with apple replant disease (13). Growth was occasionally inhibited by PGPR in our trials and in other studies (8,29) with different crops. Factors contributing to negative or static responses by PGPR are not known (4) but have been a major obstacle in the development of PGPR for commercial use (7).

Reductions in total root-associated fungi with specific decreases in *Cylindrocarpon* spp. supports results of other research concerning the effects of PGPR on the rhizosphere microflora (20,29,34). The control of *C. destructans* by PGPR may be significant because it is reported as a pathogen of many plants worldwide (3) and because New York isolates were pathogenic on apple seedlings (9). This species is considered a minor pathogen of apple roots and may be responsible for growth reductions similar to those caused by *C. lucidum*, another low-grade root-infecting fungus of apple (14). Although we found no detectable reduction in gram-positive bacteria as reported in a previous study (20), the use of more sensitive methods to assess the prokaryotic rhizosphere microflora may identify effects on specific groups of bacteria.

Increased initiation of active nodes may have been due to exogenous growth factors produced by PGPR, thereby increasing plant growth. There have been several reports of hormonal effects by bacterial inoculants, but this mechanism has been difficult to confirm (7). Numerous strains have been shown to produce auxins, cytokinins, and gibberellins in culture (5,11,12,24). Strains such as *Pseudomonas* spp. that are capable of becoming established in the rhizosphere are likely to produce hormonal effects on the host (7). Such strains could produce low levels of auxins that are stimulatory to root growth and also may evoke increased host ethylene production that is biostatic to soil microorganisms (24). A recent study (22) showed that several PGPR strains produced IAA in vitro at concentrations relatively lower than nonstimulatory and deleterious strains. There is also recent evidence of PGPR production of gibberellins by a strain that increased apple shoot growth (11) and by a strain that enhanced potato stolonization (21). Increased numbers of active nodes on apple may have been induced by hormones or by PGPR strains protecting the developing nodes from deleterious fungi and bacteria.

In one greenhouse experiment, mixtures of rifampicin-resistant marked PGPR promoted growth but the unmarked mixture did not. This difference may be a result of the methods used to prepare the inoculum. Unmarked inoculum was prepared from a single colony subculture of each strain, whereas marked inoculum was prepared by mixing 20 single colony subcultures of each strain. Subsequent testing has shown that single-colony subcultures may spontaneously mutate and lose in vitro antibiosis ability and become ineffective as PGPR (9). Similar problems with other PGPR have been discussed (30) and represent a main concern for commercial development.

The most successful method for obtaining apple rootstock PGPR was to isolate predominant rhizosphere bacteria and screen them for broad-spectrum antibiosis against apple root-infecting fungi. PGPR strains Hi, S3-1-2, and Ro were selected in this manner. Ro is a gram-negative, oxidase-negative, nonfluorescent bacterium belonging to Enterobacteriaceae, and S3-1-2 and Hi are fluorescent *Pseudomonas* spp. (9). Broad-spectrum antibiosis has not always correlated well with PGPR activity (6,7,8), but it is still widely used as a method for strain selection.

Growth promotion by rhizobacteria shows promise for increasing plant growth and crop yields in the future. In addition to the previous reports of bacterization on annual crops, we now report the ability to increase growth of perennial crops in the nursery. Although the success of our strains may be related to effects on deleterious root fungi, a closer phenotypic characterization of effective PGPR strains is needed to identify traits that correlate with successful growth-promoting ability under varying conditions. The selection or genetic engineering of strains that are adaptable to fluctuations in soil moisture, temperature, aeration, pH, and antibiotic-producing ability may increase the utility of PGPR. The need for more thorough studies on the nature and stability of antibiosis by PGPR strains is also apparent. Such knowledge could lead to improvements in methods of screening for PGPR and aid in the genetic improvement of strains.

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