Effect of Rhizobacteria and Metham-Sodium on Growth and Root Microflora of Celery Cultivars

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ABSTRACT

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Treatment of celery transplants with several rhizobacterial strains or fumigation of soil with metham-sodium promoted early plant growth and increased yield in fields infested with *Fusarium oxysporum* f. sp. *apii.* Results of both bacterial and chemical treatments varied substantially among planting times and in successive years. Other factors affecting results were the cultivar and bacterial strains that were tested. *Pseudomonas aureofaciens* strain PGS 12 caused significant increases in early plant growth in three of three trials and in yield in two of three trials with cultivar Fordhook. Increases in early plant growth or yield, however, were not obtained when cultivars Tall Utah 52-70 HK (four trials) and Tall Utah 52-70 R (two trials) were treated with PGS 12. One of the trials contained all three cultivars in a factorial design. *Bacillus* sp. X2.2 caused increases in early plant growth and yield with cultivar Fordhook in one trial and had a significant interaction with all three cultivars in the combined trial. Fumigation with metham-sodium resulted in harvest weight increases varying from 18 to 75% with the cultivar Fordhook in different seasons, and from 18 to 81% among cultivars planted at the same time. Early-season plant growth promotion did not necessarily correlate with shoot weight of plants at harvest. Some qualitative and quantitative differences in fungal microflora on roots were observed in the different bacterial and chemical treatments, but, in general, the detection methods for evaluating the seasonal succession of microflora under field conditions were too insensitive and labor-intensive to conclusively relate plant growth promotion to the presence or absence of specific microorganisms, including *F. o. apii.*

Additional keywords: bacterization, biological control, PGPR.

Fusarium oxysporum f. sp. *apii* (R. Nelson & Sherb.) Snyder & Hans. causes serious losses in yield and quality of celery (*Apium graveolens* L.). It attacks the fibrous root system and spreads through the xylem into the crowns. The initial symptom is a retardation of growth, or dwarfing, usually followed by yellowing of the foliage. Symptom expression is temperature-dependent, with incidence high in summer and low in winter. Resistant cultivars lasted for about two decades when a new race of the pathogen developed (12). Chemical control has not been effective or economically feasible (7).

Laboratory and greenhouse studies have shown that certain fluorescent pseudomonads are highly antagonistic to Fusarium wilt pathogens (16,22,24). Some bacteria have reduced Fusarium wilt of celery in neutral-to-alkaline soils in greenhouse studies (20). Because California has soils that suppress other formae speciales of wilt pathogens (3,22,23), biological control of Fusarium wilt of celery was explored as an alternative control measure.

We report here on field tests of several bacterial strains during two growing seasons with three celery cultivars. We also investigated the effect of selected rhizobacteria and soil fumigation with metham-sodium on root colonization of celery cultivars by fungal microflora. Preliminary results of this work were reported (4–6).

MATERIALS AND METHODS

Source of rhizobacteria and celery cultivars. Nine bacterial strains were from Allelix Inc., Mississauga, Ontario, Canada, courtesy of J. Kloepper; they had been selected for plant growth promotion of canola (rapeseed) (*Brassica campestris* and *B. napus*) (15). Eleven rhizobacterial strains were from Plant Genetic Systems, Gent, Belgium, and had been selected on the basis of in vitro antibiosis against a broad spectrum of plant-pathogenic fungi. Other bacteria used in these investigations had been isolated from roots of various crops grown throughout California. Gram-negative strains were identified with API 20 E test strips (Analytap Products, Ayerst Labs Inc., Plainview, NY) and by additional standard methods (11). Twenty-seven strains were identified as follows: fluorescent pseudomonads, 14; *Pseudomonas cepacia*, five; *Serratia liquefaciens*, four; and one each of *S. plymuthica, Arthrobacter citreus, Erwinia herbicola*, and *Bacillus* sp.

One or more of the following celery cultivars were used in the experiments: Fordhook (W. Atlee Burpee Co., Warminster, PA); Tall Utah 52-70 R (Pybar Seed Co., Salinas, CA); and Tall Utah 52-70 HK (Arco, Barstow, CA). In resistance evaluations, the first two cultivars were rated highly susceptible to *F. o. apii*, whereas the last one was considered moderately susceptible (19).

Biological and chemical treatments. Bacterial strains were grown for 48 hr at 28 C on Pseudomonas agar F (PAF) (Difco Laboratories, Detroit, MI) or 10% tryptic soy agar (TSA) (Difco), suspended in 0.1 M $MgSO_4$, and 1 L (about 5×10^8 colony-forming units [cfu] per milliliter) was applied as a drench to each flat of 4-wk-old celery seedlings growing in vermiculite in the greenhouse at 26 ± 2 C. Treatments were repeated after 2 wk, and the plants were placed for 1 wk in a lathhouse for hardening. The celery was fertilized weekly (Terr-o-vite, 15-15-15, Chacon Chemical Corp., CA).

Metham-sodium (Wilbur-Ellis, Fresno, CA) was applied to the field 14 days before planting at a rate of 0.47 L per 4.5 m^2 bed and watered in to a depth of approximately 12 cm.

Fertilization, irrigation, and weed control. Fertilizer (16-20-0) was applied at 45 kg of N/acre in two bands 10 cm deep, each 15 cm off the center of the bed. In addition, liquid nitrogen (4.5 kg N/acre) was added during each irrigation. Celery plants were sprinkler irrigated for the first week, then watered through T-tape drip irrigation tubes (T-Systems Corp., San Diego, CA). Irrigation was scheduled to maintain soil water potential at about −0.1 to −0.4 bars at a soil depth of 12.5 cm (monitored by tensiometers).

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For weed control, prometryn was applied at 16.7 kg a.i./ha 2 wk after transplanting.

Spring 1986 field trial with cultivar Fordhook. Although all 27 bacterial strains were included in the first field trial to determine their effects on growth of celery, the primary purpose was to test strains PGS 12, PGS X2.2, and PGS 9.2 of *Pseudomonas aureofaciens*, *Bacillus* sp., and *P. cepacia*, respectively. These three strains were selected for field trials on the basis of greenhouse screens that identified bacteria that enhanced growth of celery and other plants. Strain PGS 12 was the most highly rated strain. The screening methods were similar to methods of Burr et al (8) and Kloepper et al (16). The additional strains were included because field trials often reveal effects not observed in the greenhouse. The trial was done at the University of California South Coast Experimental Field Station, Irvine, CA, in a field that had been infested with the Fusarium wilt pathogen 6 yr before and had been cropped exclusively with celery. Soil type was a San Emigdio sandy-loam: 12% clay, 12.5% silt, 75.5% sand, 0.45% organic matter, pH 7.6, EC 1.3 millimhos/cm, 296 μg/g N, 14.5 μg/g P, 212 μg/g K, 7.2 me/L Ca, 2.9 me/L Na, 1.8 me/L Mg, 38 μg/g Mn, 46 μg/g Fe, 1.00 μg/g Zn, 0.5 μg/g Cu (Mn, Fe, Zn, and Cu were DTPA extracted).

Bacteria-treated seedlings of celery cultivar Fordhook were transplanted into the field in 3.5 × 0.6-m beds on 1-m centers, with 18 cm between plants. There were 36 plants per bed in two rows. A randomized complete block design was used with five replicates. To determine bacterial colonization at planting time, 1–2 g (fresh weight) root samples were taken from 15 seedlings treated with *P. aureofaciens* PGS 12 and from the untreated control. They were triturated in 0.1 M phosphate buffer, and 10-fold dilutions were plated on PAF plus 0.1 g of $FeCl_3$. This strain was chosen because it produced a distinctive orange pigment on PAF, which allowed differentiation from other bacteria.

The height of 10 randomly selected plants per replicate was measured 6 wk after planting. Preliminary studies had determined that early plant growth promotion could be detected by measuring either height or fresh weight of tops. Later, top weight was determined to be a slightly more accurate measure of growth. We did, however, rely on plant height when measurements were made during the growing season and there were not enough plants to sacrifice. At harvest, the stalks were removed from the roots and weighed, and total stalk weight was recorded for each replicate.

Two randomly selected plant root systems per replicate of the treatments PGS 12, metham-sodium, and the untreated control were collected for a comparison of fungal microflora. Roots were washed in tap water, blotted dry, pooled for each replicate, and cut in 2- to 3-cm pieces. Fifty centimeters of randomly selected root pieces was placed on each of 1.5% water agar (WA), Komada's medium (KM) (18), and cellophane extract-pectate agar (CEPA) (14) and incubated at 24 C. *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn were assayed on water agar plates and identified by their distinctive hyphal growth after 1 day (25). KM and CEPA plates were incubated under fluorescent lights and examined between 7 and 14 days. Orange colonies of *Fusarium* spp. were counted on KM; pathogenicity tests showed that about 90% of these colony types were *F. o. apii* (data not shown). Colonies on CEPA were distinguished by colony morphology. Several representatives of each colony type were transferred to potato-dextose agar (PDA) and identified.

Summer 1986 field trials: ***Testing of PGS strains 12 and 9.2 and metham-sodium on cultivar Tall Utah HK.*** In summer 1986, *P. aureofaciens* PGS 12, *P. cepacia* PGS 9.2, and metham-sodium were tested on cultivar Tall Utah 52-70 HK. One trial was at the same location as the spring 1986 trial, and the other was in a commercial field near Salinas, CA. The latter field was infested naturally with *F. o. apii* and had been cropped to celery the previous year. The soil was a clay loam, pH 7. Both trials were designed as randomized complete blocks with six replicates and 40 plants per replicate. Preparation of bacterial suspensions and application to seedlings and soil fumigation with metham-sodium were as previously described. After 3 mo, celery was harvested, and top fresh weights were recorded.

Cultivar field trials, fall 1986 and spring 1987. Four bacterial strains that had performed best in the spring 1986 field trial were tested on three celery cultivars at South Coast Field Station. In fall 1986, each cultivar was evaluated in a separate experiment that was designed as a randomized complete block. There were six replicates per treatment and 40 plants per replicate. Preparation of bacterial suspensions and application to seedlings and soil fumigation with metham-sodium were as previously described. The growth of cultivars Fordhook, Tall Utah 52-70 R, and Tall Utah 52-70 HK was measured 4, 5, and 6 wk after planting, respectively. Five plants from five of six replicates of each treatment were removed carefully from the soil, roots were removed, and top dry weights were determined.

Roots from the five plants sampled in each replicate were pooled, washed in tap water, and divided into two groups. The roots in one group were cut into 5-cm segments, and a total of 100 cm per replicate was placed on 1.5% water agar to determine colonization by *Pythium* and *Rhizoctonia*. The other group of roots was surface-disinfested with 0.26% NaOCl for 5 min and washed several times with sterile water, and 200 cm of root segments per replicate were plated on both KM and CEPA. After 4 mo, the celery was harvested and the mean top fresh weight per plant was determined for each replicate.

In spring 1987, the three cultivars and six treatments were combined in a randomized complete block design with six replicates per treatment-cultivar combination. Seedling heights of each cultivar were measured 7 wk after planting. Ten soil cores per replicate were taken 4, 8, and 12 wk after planting for analysis of root microflora of selected treatments. A 2.1-cm-diameter Oakfield tube (Oakfield Apparatus Co., Oakfield, WI) was pushed vertically 30 cm deep into the soil approximately 2 cm from the plant base. Soil cores from each replicate were composited and placed in a 2-L beaker. Tap water was added, and the resultant slurry was filtered through three layers of cheesecloth to catch the roots, which were washed free of soil and resuspended in sterile water. Roots were deposited onto a sterile metal screen in a Buchner funnel. One hundred centimeters of root segments from each replicate was placed on a root-plating medium (RPM) containing 35 ml of V-8 juice, 1 g of $CaCO_3$, 0.2 g of penicillin G, 0.2 g of chlortetracycline, 0.2 g of streptomycin sulfate, 0.1 g of kanamycin sulfate, 2.0 ml of tergitol 7 anionic, 10 g of PDA, and 11 g of Bacto agar (Difco) in 1 L of deionized water. Plates were incubated for 4 days at 22 C under fluorescent light. Fungi were counted and identified under low-power magnification. Samples were taken to verify identification under higher magnification, or after culture on PDA. After 3 mo, celery was harvested and top fresh weight was determined.

Greenhouse tests. Because of their performance in promoting growth of celery in the spring 1986 field trial, *P. aureofaciens* PGS 12 and *P. cepacia* PGS 9.2 were tested for their potential to reduce root infections by *F. o. apii* in greenhouse tests. *P. fluorescens* TL 13 was selected for comparative purposes because it did not affect celery growth in the first field trial. Celery seed (cultivar Fordhook) was surface-disinfested for 2 min in 0.525% NaOCl, washed several times with sterile water, and seeded into trays containing vermiculite. After germination, seedlings were fertilized weekly with commercial nutrient solution (Terr-o-vite, 15-15-15, Chacon Chemical Corp., CA). Plants were removed carefully 6 wk after seeding, and roots were placed in bacterial suspensions or sterile water (control) for 30 min. Suspensions were prepared by growing the bacterial strains in nutrient broth for 48 hr at 24 C; cells were washed with 0.01 M $MgSO_4$ and diluted to about 10^9 cfu/ml. After treatment, each seedling was planted into a plastic cone (15 cm long × 2.5 cm diameter; Ray Leach Cone-tainer, Canby, OR) containing field soil that had been fumigated and artificially infested with *F. o. apii*. The experiment was designed as a randomized complete block with five replicates and two seedlings per replicate. The soil was collected from the top 10 cm of a field at the South Coast Experimental Field Station and screened through a 10-mm-pore sieve. The soil was fumigated with metham-sodium (30 ml/0.056

m^3 of soil) and covered with a plastic tarpaulin for 2 days. After removal of the tarpaulin, the soil was allowed to aerate for 3 wk. Chlamydospores of *F. o. apii* were produced as described (20) and mixed into the soil to give a population density of approximately 500 cfu/g of soil. After 4 wk in the greenhouse at 25 ± 2 C, plants were removed from the soil, and roots were washed carefully and cut in 2- to 3-cm lengths. These were disinfested in 0.525% sodium hypochlorite for 1 min and placed on KM. For each replicate, 40 cm of randomly selected root segments were plated. The plates were incubated at 24 C for 7 days before the root area colonized by *F. oxysporum* was measured.

Data analyses. Data were subjected to analysis of variance, and least significant differences were calculated for mean separation. In the spring 1987 trial, data for bacterial and fumigation treatments were separately analyzed and subjected to Duncan's multiple range test and orthogonal comparisons after an analysis of variance.

RESULTS

Spring 1986 field trial with cultivar Fordhook. When inoculated onto roots of transplants, PGS strains 12, 9.2, and X2.2 caused significant increases in early plant growth (top heights) and/or yield (21–32%) of celery cultivar Fordhook compared with the untreated control (Table 1). Some other strains caused growth increases that barely missed significance at $P = 0.05$. Of these, only All 31-12 was selected for further trials.

Fumigation with metham-sodium resulted in yield improvement of 74%. The population density of *P. aureofaciens* PGS 12 on roots of transplants at planting was 10^6 cfu/g of root. Densities of other strains were not determined.

There was a significant reduction ($P = 0.05$) in population density of *Cylindrocladium* spp., *Fusarium* spp., *Trichoderma* spp., and *F. o. apii* on roots of plants grown in fumigated soil compared with those grown on roots in unfumigated soils (data not shown). Similarly, roots of plants treated with PGS 12 had reduced population densities of *Trichoderma* spp. and *F. o. apii* ($P = 0.1$) but a greater population density of *Fusarium* spp. and *Cylindrocladium* spp. ($P = 0.1$) compared with the roots of the untreated control; population densities of *Pythium* and *Rhizoctonia* spp. were very low in all treatments, including the untreated control (data not shown).

Summer 1986 field trials: *Testing of PGS strains 12 and 9.2, metham-sodium on cultivar Tall Utah 52-70 HK.* In contrast to the previous results, bacterial treatments did not significantly affect yield of this cultivar, which is moderately susceptible to *Fusarium*, compared with the untreated control in both field trials (data not shown). However, soil fumigation caused significant ($P = 0.05$) yield increases of 23% at South Coast Field Station and 76% at Salinas.

Cultivar field trial, fall 1986. Only cultivar Fordhook treated with PGS 12 showed a significant early season increase in top dry weight (Table 2). At harvest, however, the only significant increase in yield (18%) occurred with strain All 31-12 on Tall Utah 52-70 R (Table 2). Soil fumigation with metham-sodium resulted in significant yield increases ranging from 18 to 81% (Table 2) with all cultivars.

Analysis of microflora isolated from roots of plants growing 4–6 wk in the field indicated great variation in the incidence of fungal colonization among replicates of the same treatment; however, some differences were noted among cultivars and treatments (Table 3). The number of infections by *F. o. apii* was very low in the untreated controls of cultivar Fordhook and cultivar Tall Utah 52-70 HK and was at a moderate level in the highly susceptible Tall Utah 52-70 R. *P. ultimum* was not detected from roots of cultivar Fordhook in fumigated soil but was isolated from the roots of the other two cultivars. Despite relatively low frequency of infection and high variability among replicates within treatments, a statistically significant difference in *P. ultimum* on roots was detected with the treatment All 31-12, but only with cultivar Fordhook. Some of the treatments appeared to cause an increase in the incidence of colonization of *Penicillium* spp.

Cultivar field trial, spring 1987. Seven weeks after planting, metham-sodium and nearly all of the bacterial treatments significantly increased plant growth compared to that of the untreated control (Table 4). By harvest, however, only the bacterial strains PGS 12 and PGS X2.2 caused significant increases in yield, and only with cultivar Fordhook (Table 4). The analysis of variance indicated that no interaction was found between bacterial treatments and cultivars. However, orthogonal comparisons revealed a significant interaction between all cultivars and PGS X2.2. No significant metham-sodium × cultivar interaction was found.

With the exception of the metham-sodium treatment, which generally resulted in a significant reduction in the population densities of *F. o. apii* and other fungi on plant roots, the analysis of population density did not reveal any major differences among treatments (Table 5) that could account for the differences in plant growth promotion and yields.

Greenhouse tests. Colonization of celery seedling roots of the cultivar Fordhook by *F. o. apii* was greatly reduced by both

TABLE 1. Effect of soil fumigation and seedling treatment with selected rhizobacteria on growth of celery (cultivar Fordhook) in a field infested with *Fusarium oxysporum* f. sp. *apii*; spring, 1986

Treatment[a]	$\bar{X}$ Plant height after 8 wk (cm)[b]	$\bar{X}$ Yield after 11 wk (kg)[c]
Untreated control	23.9	5.56
Metham-sodium	28.5*[d]	9.70*
PGS 9.2	24.9	6.75*
PGS X2.2	24.3	6.84*
PGS 12	26.3*	7.33*
LSD ($P = 0.05$)	2.4	1.14

[a]Metham-sodium was applied as a soil drench at 0.47 L/4.5 m^2 14 days before planting. Bacterial treatments were applied as a drench to greenhouse-grown seedlings 4 and 6 wk after seeding (1 L per flat, approximately 5×10^8 cfu/ml).
[b]Based on 10 randomly selected plants per replicate, five replicates per treatment.
[c]Based on the total fresh top weight of 18 plants from each replicate, five replications per treatment.
[d]* = Significantly different from control ($P = 0.05$).

TABLE 2. Effect of soil fumigation and preplant treatment of seedlings with selected strains of rhizobacteria on growth and yield of three celery cultivars in a fall field trial, 1986

	$\bar{x}$ Growth (g top weight/plant) of cultivar					
	Fordhook		Tall Utah 52-70 R		Tall Utah 52-70 HK	
Treatments[a]	Early season[b]	Harvest[c]	Early season[b]	Harvest[c]	Early season[c]	Harvest[c]
Untreated control	8.0	778.7	20.8	502.7	19.2	765.0
Metham-sodium	10.6	922.0*	30.6*	908.8*	31.9*	990.5*
PGS 9.2	10.3	816.2	23.4	562.2	18.1	789.0
PGS X2.2	10.2	752.8	25.1	573.8	13.3	766.2
PGS 12	12.2*[d]	829.7	23.3	502.7	13.5	728.7
All 31-12	10.7	777.7	28.0	594.8*	18.1	802.8
LSD ($P = 0.05$)	3.5	106.4	8.1	83.1	7.0	102.1

[a]Metham-sodium was applied as a soil drench at 0.47 L/4.5 m^2 14 days before planting. Bacterial treatments were applied as a drench to greenhouse-grown seedlings 4 and 6 wk after seeding (1 L per flat, approximately 5×10^8 cfu/ml).
[b]Top weights were recorded after 4 (Fordhook), 5 (Tall Utah 52-70 HK), and 6 wk (Tall Utah 52-70 R), respectively. Each value based on a sample of five plants from each of five replicates.
[c]Yield was determined 4 mo after planting. Each value is based on 40 plants per replicate and six replicates per treatment.
[d]* = Significantly different from control ($P = 0.05$). Comparisons of early-season top weight should not be made among cultivars, since samplings were taken at different times.

TABLE 3. Effects on fungal colonies/200 cm root of soil fumigation and preplant treatments of seedlings with selected strains of rhizobacteria on fungal microflora of roots of celery cultivars Fordhook (F), Tall Utah 52-70 HK (HK), and Tall Utah 52-70 R (R) in a fall field trial, 1986[a]

Fungi	Media[b]	Cultivar	Treatment[c]					
			Control	Metham	PGS 12	All 31-12	PGS 9.2	PGS X2.2
Pythium ultimum	WA	F	7	0*[d]	4	3*	ND[e]	ND
		HK	6	4	6	6	2	7
		R	4	4	3	4	3	5
Fusarium oxysporum f. sp. *apii*	KM	F	1	1	1	1	ND	ND
		HK	1	0	2	2	1	2
		R	6	0*	5	8	5	6
Penicillium spp.	CEPA	F	4	8	9	8	ND	ND
		HK	4	3	10	14	56*	14
		R	26	51*	66*	26	14	18
Fusarium spp.	CEPA	F	13	9	3	6	ND	ND
		HK	14	2	9	9	10	13
		R	25	11	12	19	20	20
Scopulariopsis spp.	CEPA	F	5	0	3	3	ND	ND
		HK	6	1*	4	3	2*	4
		R	18	1*	9	11	13	4*
Other fungi	CEPA	F	4	3	3	2	ND	ND
		HK	5	3	5	4	2	4
		R	7	3*	1*	4*	4*	3*
Total fungi	CEPA	F	26	20	18	19	ND	ND
		HK	29	9	28	30	70*	35
		R	76	66	88	60	51*	45*

[a]Samples were taken from Fordhook, Tall Utah 52-70 HK, and Tall Utah 52-70 R at 4, 5, and 6 wk after planting, respectively. Therefore, data are presented for comparisons to be made among treatments of a specific cultivar and not for comparisons among cultivars.
[b]Water agar (WA), Komada's medium (KM), and cellophane extract-pectate agar (CEPA).
[c]Metham-sodium applied 14 days before planting as soil drench at 0.47 L/4.5 m^2; bacteria applied twice as a drench (1 L per flat, about 5×10^8 cfu/ml) to seedlings growing in the greenhouse.
[d]* = Significant difference compared with untreated control at $P = 0.05$ for the specific cultivar.
[e]ND = Not determined.

TABLE 4. Effect of soil fumigation and preplant treatment of seedlings with selected strains of rhizobacteria on growth and yield of three celery cultivars in a spring field trial, 1987

Cultivars and treatments[a]	$\bar{x}$ Early season plant height (cm)[b]	$\bar{x}$ Yield (g top wt/plant)[c]
Tall Utah 52-70 HK		
PGS X2.2	26.9 ab[d]	632.6 a
PGS 9.2	26.0 abc	598.9 abc
Metham-sodium	27.4 abcd	683.9 a
PGS 12	25.3 bcd	581.0 abcd
All 31-12	24.6 cde	555.2 bcd
Untreated control	23.3 efg	619.2 ab
Fordhook		
PGS X2.2	22.7 efg	628.4 a
All 31-12	23.0 efg	572.4 abcd
Metham-sodium	22.3 fg	684.2 a
PGS 12	22.5 fg	586.3 abc
PGS 9.2	21.3 gh	539.6 cd
Untreated control	19.8 h	520.8 d
Tall Utah 52-70 R		
PGS X2.2	23.1 efg	420.3 e
All 31-12	23.0 efg	418.4 e
PGS 12	22.7 efg	411.9 e
PGS 9.2	22.4 fg	410.9 e
Untreated control	20.3 h	395.4 e
Metham-sodium	23.7 def	541.0 bc

[a]Metham-sodium (0.47 L/4.5 m^2) was applied as a soil drench 14 days before planting. Bacterial treatments were applied twice as a drench (1 L per flat, approximately 5×10^8 cfu/ml) to seedlings growing in the greenhouse.
[b]Plant height was measured 7 wk after planting.
[c]Each value is based on 40 plants per replicate and six replicates per cultivar-treatment combination.
[d]Means followed by the same letter do not differ according to Duncan's multiple range test ($P = 0.05$).

PGS 12 and PGS 9.2, whereas a non-growth-promoting fluorescent pseudomonad TL 13 used as a control was ineffective. Two repetitions of this test yielded similar results. Since error variances of two trials were homogeneous, an overall analysis of variance was performed on pooled data. A total of 40 cm of roots from each of 10 plants per treatment were plated on KM. The mean length of colonized roots from the untreated control and from plants treated with PGS 9.2, PGS 12, and TL 13 was 24.0, 12.4, 3.5, and 22.0 cm, respectively. The values for PGS 9.2 and PGS 12 were statistically significantly different ($P = 0.05$) from those for the control and TL 13.

DISCUSSION

The most significant finding from this research was the increased early-season growth and yield responses of celery cultivars to specific rhizobacteria and fumigation by metham-sodium. Comparison of rhizobacterial treatments with metham-sodium provided perspective on the consistency of biological versus chemical treatments. The magnitude of the growth response varied greatly among plantings and in successive years for both the biological and chemical treatments, but the fumigant provided the most consistent results. In our opinion, it was not the time of planting per se that affected results, especially with bacterial treatments, but the differences in growing conditions that may occur from one season to the next. We attribute the lack of results from bacterial treatments in the 1986 fall planting to the severity of Fusarium wilt and the unusually hot temperatures that occurred. However, a precise determination of the effects of planting times, growing conditions, and the role of plant genotype (as discussed later) on the efficacy of bacterial treatments and chemicals can be determined only by years of field trials.

The response of the cultivars to the fumigant was similar, whereas responses to the bacterial treatments appeared to be genotype related. With cultivar Fordhook, the treatment of transplants with PGS 12 resulted in significant increases in early plant growth and yield in three of three and two of three trials, respectively, whereas no effect was detected in cultivar Tall Utah 52-70 HK or Tall Utah 52-70 R in four and two trials, respectively. Although location effects cannot be excluded for the 1986 fall trial because of the experimental design, comparison of three cultivars in the spring trial of 1987 confirmed the previous indications that the cultivars responded differentially to the bacteria. Plant growth response from treatments with PGS X2.2

TABLE 5. Effects (cfu/10 cm root) of soil fumigation and preplant treatments of seedlings with selected strains of rhizobacteria on fungal microflora of celery roots in a spring field trial, 1987

Fungi	Media[a]	Sample taken (weeks after planting)	Cultivars and treatments[b]											
			Fordhook				Tall Utah 52-70 HK				Tall Utah 52-70 R			
			Control	Metham	PGS 12	PGS 9.2	Control	Metham	PGS 12	PGS 9.2	Control	Metham	PGS 12	PGS 9.2
Fusarium oxysporum f. sp. *apii*	KM	4	7	1*[c]	7	7	8	2*	6	8	9	3*	6	8
		8	4	3	5	5	4	2	4	5	6	2*	4*	5
		12	9	4*	8	8	8	5*	8	7	7	3*	7	7
Fusarium spp.	RPM	8	14	10	16	12	15	10	13	14	16	8*	15	15
		12	12	9	11	11	16	10*	17	16	16	11*	16	16
Pencillium spp.	RPM	8	3	2	4	2	4	5	3	4	5	1*	4	2*
		12	5	8	4	4	7	7	6	4	8	9	6	4
Alternaria spp.	RPM	8	1	1	1	1	2	0*	1	0*	1	1	2	1
		12	2	2	3	2	1	2	1	1	2	2	2	1
Aspergillus spp.	RPM	8	1	0	2	1	1	0	1	2	1	0	1	1
		12	2	1*	2	3	1	0	2	2	2	1	1	3*
Total fungi	RPM	4	14	8*	14	12	14	6*	16	14	12	7*	15	8*
		8	21	15	26	22	30	18*	34	35	35	16*	33	33
		12	28	24	28	28	27	22*	28	28	29	25	26	26

[a]Komada's medium (KM), root plating medium (RPM)).
[b]Metham-sodium applied as soil drench at 0.47 L/4.5 m^2 14 days before planting; bacteria applied twice as a drench (1 L per flat, approximately 5×10^8 cfu/ml) to seedlings growing in the greenhouse.
[c]* = significantly different from control ($P = 0.05$). Comparisons are to be made only within each cultivar and not among cultivars.

was greatest with the cultivar Fordhook, even though it significantly interacted with all cultivars in one trial. This is the first field evidence suggesting that plant genotype affects the efficacy of bacterial growth promoters, although a similar effect was found in greenhouse experiments with bacteria on different wheat and pea cultivars (1).

A major objective of this investigation was to develop a biological control for *F. o. apii*. This was considered a formidable challenge because of the nature of the disease; infection of the relatively unprotected, continuously advancing root tips is sufficient to allow vascular colonization. However, bacterial antagonists have reduced root colonization by *F. oxysporum* (26) and germination of its propagules (10,21,22,24,26). Since multiple infections of the pathogen are necessary for severe disease occurrence (13), it was thought that any significant reduction in the number of infections by *Fusarium* would have an effect on disease development. Accordingly, our experiments showed that *P. aureofaciens* PGS 12 and *P. cepacia* PGS 9.2 suppressed root colonization by *F. o. apii* in greenhouse-grown plants and in the early season in some of the field treatments. Neither the bacterial antagonists nor metham-sodium, however, seemed to have much effect on the expression of disease symptoms by harvest. However, we believe the increased yields that resulted from the use of metham-sodium and bacterial antagonists were caused, at least in part, by some reduction of *F. o. apii* and the population sizes of minor or cryptic pathogens, thereby enhancing root health (9,17,27).

There has been a tendency toward unrealistic expectations when evaluating the performance of biological agents. Efficacy of such agents is usually measured in terms of plant response at harvest, rather than their effect on a specific pathogen or pathogens. Use of chemicals often does not result in yield increases, especially when disease pressure is low or when nontargeted pathogens affect plant growth. Similar results are found with biocontrol agents. The efficacy of both chemicals and biologicals are affected greatly by physical and biological properties of the soil. In our experiments, much of the variability in results probably was caused by differences in the composition of the microflora in soil and subsequent colonization patterns on roots.

A great effort was made to detect alterations in root microflora among cultivars and between different planting dates and to relate growth responses to the occurrence of particular root microflora. Although some differences in fungal colonization were noted, degree of resolution was too low to convincingly relate plant growth response to the presence or absence of any particular organism.

These investigations reflect the inadequacy of techniques for monitoring microflora on roots during a season and the need to develop more sensitive and less labor-intensive methods. Fibrous root systems are difficult to monitor microbiologically because of the great variation in microflora among roots of different ages. Sample sizes are so large that it is not possible to process more than several treatments at a time, resulting in assaying of plants of different ages. Also, roots of the same age must be sampled (e.g., root tips and root segments from a defined distance distal from the seed or crown) either by carefully removing the plant with its roots from the soil, or excavating at the side of the furrow and excising roots as was done with potatoes (2).

Our study shows the potential for using biologicals to promote plant growth. However, it also reaffirms that, for effective use of microbial inoculants, we need more knowledge on several fundamental problems ranging from methods of applying inoculum to the understanding of mechanisms affecting colonization and antagonism in the rhizosphere.

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