Interactions of *Pseudomonas fluorescens* strain E6 with Ornamental Plants and Its Effect on the Composition of Root-Colonizing Microflora

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ABSTRACT

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Pseudomonas fluorescens strain E6 increased growth of carnation, stock, sunflower, vinca, and zinnia when inoculated onto seeds or rooted cuttings. Fresh top weights in E6-treated plants 3–4 wk after inoculation were 18–41% greater than those of controls. However, seed inoculation with E6 frequently restricted growth of balsam, marigold, and morning glory by 7–13% and had no affect on cleome, nasturtium, and scarlet runner bean. Enhancement of zinnia growth by inoculation with E6 was not influenced by soil type. The treatment increased top weight in 19 of 23 experiments in four field soils with varying texture and pH. Population sizes of E6 on zinnia roots, however, differed among the soils. Growth promotion in zinnia by strain E6 was related to a change in the composition of root microflora and a reduction in the deleterious effects of minor pathogens. Colonization of zinnia roots by *Penicillium* spp. was less following inoculation with E6, whereas colonization by *Fusarium* spp. was greater. The treatment did not change the total number of fungi or bacteria on the roots. When zinnias were planted into soils infested separately with 10^4 to 10^5 propagules of *Eupenicillium javanicum, Penicillium janthinellum, P. citreonigrum*, or *P. citrinum* (each isolated from zinnia roots) per gram of soil, top weights of plants after 3 wk were reduced by 23–57%. Inoculation of zinnia seed with E6 prior to planting in soils infested with *E. javanicum, P. janthinellum*, or *P. citreonigrum* resulted in reduced root colonization by *Penicillium* spp. and in plant growth similar to that in noninfested soil. Zinnias were not affected when planted into soils infested with other root-colonizing fungi.

Additional key words: antagonism, biological control.

Plant-growth-promoting rhizobacteria (PGPR) colonize plant roots and cause increases in plant growth and yield (4,8,10,21). The specific mechanisms by which these bacteria affect plant growth, however, are not clear. The elaboration of growth-stimulating phytohormones by bacteria was suggested as one possible mechanism for growth promotion (1,3). Some PGPR produce detectable indole-3-acetic acid; in general, however, a negative correlation exists between the concentrations of indole-3-acetic acid produced by a strain in vitro and the influence of the strain on root elongation (11). Results of several studies (9,19,20) suggest that growth promotion is related in part to the displacement of deleterious microflora, presumably "minor pathogens" as defined by Salt (14). Antibiosis against various pathogens and other microorganisms in vitro has been demonstrated with many of the PGPR (4,9,21) and with other beneficial bacteria (2,5,25). This has been related to their in vivo antagonism (9). Siderophore production also has been related to in vivo actagonism (7,16,18,28).

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Pseudomonas fluorescens Migula strain E6 was used in several studies on plant growth promotion (8,10,21) and appeared to be nonspecific in its ability to enhance growth in a variety of plant species. The purposes of this study were to determine the range of plants with which strain E6 can interact, and to study the role of soil type on the microbial-plant interactions. A further objective was to determine whether or not the bacterial strain alters the composition of root-colonizing microflora, as was found in previous studies with other PGPR strains (9,19). A preliminary report has been published (27).

MATERIALS AND METHODS

Eleven plant species representing nine families (Table 1) were tested for growth responses, including early emergence, when inoculated with strain E6. Each species was tested in three or more experiments. Seeds and the roots of cuttings were either dipped into suspensions of strain E6 (10^8 colony-forming units [cfu] per milliliter in 0.1 M $MgSO_4$) or immersed in water as controls. Depending upon the species, six to 10 seeds or three rooted cuttings were planted into 10- to 15-cm-diameter clay pots. Eight to 16 replicate pots were planted per treatment and were arranged in a randomized complete block design. The plants were maintained for 3–4 wk in a greenhouse, with daytime temperatures of 19–32 C, and were watered and fertilized daily with tenth-strength Hoagland's nutrient solution. Stand counts were taken within the first 7–10

days after planting, and the number of plants in each pot were thinned to the same numbers, usually three to five per pot. At the end of the growing period, the tops from all plants in a pot were removed and combined, and the fresh weights were determined. Student's *t*-test (hereafter called *t*-test) was performed to determine statistical significance.

The soil used in the experiments, unless stated otherwise, was nontreated Delhi sand, subgroup Typic Xeropsamments. Soil from the top 30-cm was collected and stored in plastic bags at room temperature for no longer than 2 mo; it was mixed and sieved prior to use.

Measurement of rhizosphere microfloral population densities. Root segments or entire root systems with adhering soil were transferred to sterile 0.1 M $MgSO_4$ and shaken for 30 min. The washings were serially diluted and plated on appropriate agar media.

A rifampicin-resistant strain of E6 used in all experiments was detected by plating dilutions on King's Medium B agar (KBA) amended with rifampicin (Sigma Chemical Co., St. Louis, MO) at 100 μg/ml and each of the fungicides cycloheximide (Sigma Chemical Co.) and benomyl (DuPont Co., Wilmington, DE) at 150 μg/ml. To measure population densities of other bacteria, dilutions were plated on one-fifth strength KBA or one-fifth-strength trypticase soy agar (Difco Laboratories, Detroit, MI). Both media were amended with the two fungicides.

Colony counts were recorded after incubation at 28 C for 2 days. A portion of the bacteria growing on KBA and TSA were classified for Gram-stain reaction by using Ryu's (22) potassium hydroxide technique. Fluorescent colonies of *Pseudomonas* spp. on KBA were detected by using ultraviolet light. No further identification of bacterial strains was attempted. Six to 12 replicate measurements were made for each treatment. Population counts were converted to logarithmic units prior to statistical analysis with the *t*-test or Duncan's multiple range test (12).

TABLE 1. Plant species tested for growth response to seed inoculation with *Pseudomonas fluorescens* strain E6

Species and cultivar	Family	Common name
Cleome hasslerana Chodat. 'Giant Rose Queen'	Capparaceae	Cleome
Dianthus caryophyllus L. 'White Improved Sim'[a]	Caryophyllaceae	Carnation
Dianthus caryophyllus L. 'Red Wonder Chabaud'	Caryophyllaceae	Carnation
Helianthus annuus L. 'Big Black'	Asteraceae	Sunflower
Impatiens balsamina L. 'Peppermint'	Balsaminaceae	Balsam
Ipomoea tricolor Cav. 'Heavenly Blue'	Convolvulaceae	Morning glory
Matthiola incana R. Br. 'Double Dwarf'	Brassicaceae	Stock
Phaseolus coccineus L.	Fabaceae	Scarlet runner bean
Tagetes erecta L. 'King Tut'	Asteraceae	Marigold
Tropaeolum minus L. 'Dwarf Cherry Rose'	Tropaeolaceae	Nasturtium
Vinca minor L. 'Alba'	Apocynaceae	Vinca; periwinkle
Zinnia elegans Jacq. 'Giant Double Rose Pink'	Asteraceae	Zinnia

[a] Roots of cuttings were treated.

Fungal colonization of zinnia roots was assayed by washing roots under tap water, shaking them in three volumes of sterile 1% sodium hexametaphosphate for 30 min each, and rinsing them in sterile distilled water. The distal 2-cm portion of each root was excised and aligned on cellophane extract agar (23). Approximately 120 cm of root segments were plated for each replication. There were at least six replications per treatment. The cultures were incubated at 25 C for 14 days. At 2-day intervals during this period, fungal colonies along the roots were counted and examined under a compound microscope at ×100 and ×400 for identification to genus. Hyphal and conidial transfers were made from some colonies onto potato-dextrose agar (PDA) for further identification and testing. The *t*-test was used in the statistical analysis of the data.

Effect of rhizosphere fungi on zinnia growth. Fungi isolated from zinnia roots on cellophane extract agar were cultured on PDA for 1 wk. Conidia and hyphal fragments were washed from the agar surface with sterile water and incorporated into samples of Delhi sand. After the infested soils had been stored for 1 week, population densities of the fungi were measured by plating dilutions of soil suspensions onto one-tenth-strength PDA amended with tergitol-NPX (Baker Chemical, Co., Phillipsburg, NJ) at 1 ml/L, and penicillin-D, streptomycin sulfate, and tetracycline hydrochloride, each at 150 μg/ml. Zinnia seeds treated with strain E6 or water were planted in soil infested with different fungi and grown under the greenhouse conditions described above. After 3 wk, measurements were made of the fresh top weight of the plants, rhizosphere population densities of E6, and root colonization by various fungi. The data were analyzed with the *t*-test or Duncan's multiple range test.

RESULTS

Host specificity of growth response. Plant growth promotion by strain E6 was not a general phenomenon and occurred only with certain plant species (Table 2). Fresh top weights were increased in five species when E6 was inoculated onto seed or rooted cuttings, whereas either no effect or lower top weights were noted with treatment of other plant species. Growth of zinnia, sunflower, stock, vinca, and carnation was enhanced, with increases averaging from 18 to 41% over that of untreated controls. In 11 of 14 experiments with zinnia, statistically significant increases averaging 41% were recorded. Growth of two carnation cultivars

TABLE 2. Growth response of ornamental plant species resulting from preplant inoculation of seeds or rooted cuttings with *Pseudomonas fluorescens* strain E6

Plant	Change in top weight over control (%)[w]		Proportion of experiments significant at $P \leq 0.10$[y]
	Average[x]	Range	
Balsam	− 13	− 27 to + 14	3/5
Carnation 'White Improved Sim'[z]	+ 40	+ 23 to + 53	3/3
'Red Wonder Chabaud'	+ 18	+ 7 to + 26	2/3
Cleome	+ 2	− 6 to + 8	0/3
Marigold	− 12	− 44 to + 19	4/8
Morning glory	− 7	− 23 to + 13	1/3
Nasturtium	− 2	− 9 to + 4	0/3
Scarlet runner bean	+ 3	0 to + 6	0/3
Stock	+ 19	+ 16 to + 22	2/3
Sunflower	+ 34	+ 19 to + 57	3/3
Vinca	+ 27	+ 7 to + 46	2/3
Zinnia	+ 41	+ 2 to + 146	11/14

[w] Top weight measured 3–4 wk after planting in Delhi sand with eight to 16 replicate pots per treatment and three to five plants per pot.

[x] Average from all experiments regardless of statistical significance.

[y] Statistical significance in each experiment was determined by Student's *t*-test.

[z] The roots of cuttings of cultivar White Improved Sim were treated wtih E6 at 10^8 colony-forming units per milliliter of suspension. For all other plants, seeds were treated with E6.

was greater by 18 and 40%, with the greatest growth occurring in response to inoculation of roots of cuttings.

Treatment of marigold, balsam, and morning glory with E6 depressed growth by an average of 7 to 13%. However, this did not occur consistently. Increases in growth of these three species following treatment with E6 also were recorded, although none were statistically significant. Inoculation of cleome, nasturtium, and scarlet runner bean with E6 resulted in no significant effect on growth.

No correlations could be made between increased emergence and growth promotion. There were higher stand counts from inoculation of zinnia and balsam in four of 14 and one of five trials, respectively, but these were significant only at $P = 0.10$.

Growth promotion in different soils. Statistically significant ($P < 0.10$) increases in top weight of zinnia were obtained in response to seed inoculation with strain E6 in 19 of 23 tests in samples of four different field soils (Table 3). The soil textures ranged from sand to fine sandy loam, available water capacity from 0.05 to 0.21 cm/cm soil, and pH from 4.9 to 7.2. Average increases varied from a high of 41% in Delhi sand to a low of 17% in Elder sandy loam. With three of the soils, the variation in the amount of growth enhancement among soils was less than the variation observed among experiments in the same soil.

TABLE 3. Growth promotion of zinnia in different soils as a result of seed inoculation with *Pseudomonas fluorescens* strain E6[x]

Soil; subgroup; available water capacity (cm/cm soil); pH	Experiment	Top weight (g)[y] Control	E6	Increase (%)
Delhi sand; Typic Xeropsamments; 0.05–0.06; 4.9	1	5.2	5.3	2
	2	2.9	3.0	4
	3	7.8	8.6	10
	4	2.2	2.5	14*[z]
	5	4.2	5.0	19†
	6	4.0	4.8	19†
	7	3.9	5.0	28*
	8	3.7	4.7	27*
	9	2.5	3.8	52*
	10	1.7	2.6	53*
	11	3.0	4.6	53*
	12	1.5	2.4	63*
	13	3.2	5.6	75*
	14	1.4	3.3	136*
	Mean			39
Oceano loamy sand; Alfic Xeropsamments; 0.05–0.08; 5.9	1	3.3	4.2	27†
	2	1.6	2.3	43*
	3	4.1	6.2	51*
	Mean			40
Elder sandy loam; Cumulic Haploxerolls; 0.10–0.15; 6.8	1	3.1	3.0	−3
	2	4.0	5.1	28*
	3	4.4	5.7	30†
	Mean			18
Yolo fine sandy loam; Typic Xerothents; 0.19–0.21; 7.2	1	5.2	6.9	33*
	2	4.6	6.2	35*
	3	5.6	7.9	41*
	Mean			36

[x] Seeds were immersed into either suspensions containing 10^8 colony-forming units of E6 per milliliter or into water as a control.

[y] Average eight to 16 replicate pots with three or four plants per pot. Plants were grown in a greenhouse at 19–32 C for 3–4 wk.

[z] The dagger symbol (†) and asterisk (*) indicate the increase was significant at $P = 0.10$ and 0.05, respectively, based on the *t*-test performed on top weight data.

Effect of soil type on root colonization by strain E6. Population sizes of E6 on zinnia root systems declined over a 3-wk period (Table 4). They were greatest on roots of plants grown in Yolo fine sandy loam and lowest on those grown in Delhi sand. By 3 wk after planting, E6 densities of 1.2×10^3 cfu/cm were detected on the roots of plants grown in Yolo fine sandy loam, while only 46 cfu/cm were detected on roots from Delhi sand. The differences in population densities were not caused by variations in the rate of root growth within different soils, as total root masses of plants in each soil were similar at each weekly reading.

Effect of strain E6 on microfloral populations. Total bacterial population densities detected on the roots of both E6-inoculated and water-treated control plants 3 wk after planting ranged from 1 to 6×10^5 cfu/cm and were not significantly different. No significant changes in population densities of Gram-negative bacteria, fluorescent pseudomonads, or actinomycetes in response to inoculation with E6 were detected.

Inoculation of zinnia seed with E6 resulted in signficantly greater root colonization by some genera of fungi and less colonization by others (Table 5). Species of *Fusarium* Link, *Penicillium* Link, *Gliocladium* Corda, and *Cylindrocarpon* Wollenw. were the most common fungi found on zinnia roots. Colonization by *Fusarium* spp. was 65% greater as a result of E6 inoculation. In contrast, the frequency of *Penicillium* spp. was 54% lower. Inoculation with E6 had no effect on colonization by *Gliocladium* spp. and *Cylindrocarpon* spp. Species of *Alternaria* Nees, *Aspergillus* Link, *Cephalosporium* Corda, *Dictyosporium* Corda, *Paecilomyces* Bain., *Phoma* Desm., *Trichoderma* Pers., and *Ulocladium* Preuss. individually numbered less than 5% of the total fungal population on roots and, as a group, were not affected by inoculation with E6. Inoculation with E6 did not change the total number of fungi colonizing the roots. This experiment was repeated once in the greenhouse and two other times in a growth chamber with similar results.

Inhibition of zinnia growth by rhizosphere fungi. *Penicillium citrinum* Thom., *P. citreonigrum* Dierckx, *P. janthinellum* Biourge, and *Eupenicillium javanicum* (van Beyma) Stolk & Scott (each isolated from zinnia roots) reduced the growth of zinnias (Table 6). Top weights of plants 3 wk after planting were 37–57% less when grown in Delhi sand infested with the four species of *Penicillium* separately at 10^4 to 10^5 propagules per gram than when grown in uninfested soil. No other symptoms were observed on the above-ground parts or root systems of the stunted plants. Zinnia growth was unaffected in soils similarly infested with the other fungal species (*Fusarium oxysporum* Schl., *Fusarium solani* (Mart.) Sacc., *Cylindrocarpon* sp., *Phoma* sp., and *Trichoderma* sp.) isolated from zinnia roots.

Preplant seed treatment with E6 reversed the stunting effect caused by *E. javanicum*, *P. citreonigrum*, and *P. janthinellum* (Table 7). Without bacterial treatment, top weight was lower by an

TABLE 4. Population levels of *Pseudomonas fluorescens* strain E6 detected on roots of zinnias grown in different soils following application to seed[x]

Soil	Subgroup	Available water capacity (cm/cm soil)	pH	E6 population denisty ($\log_{10}$ cfu/cm)[y] 1 wk	2 wk	3 wk
Yolo fine sandy loam	Typic Xerothents	0.19–0.21	7.2	5.20 b[z]	4.08 c	3.08 b
Elder sandy loam	Cumulic Haploxerolls	0.10–0.15	6.8	5.56 b	4.52 c	2.86 b
Oceano loamy sand	Alfic Xeropsamments	0.05–0.08	5.9	4.48 a	3.60 b	1.92 a
Delhi sand	Typic Xeropsamments	0.05–0.06	4.9	4.04 a	2.65 a	1.66 a

[x] E6 was applied to zinnia seed as a 10^8 cfu/ml suspension prior to planting. Plants were grown in the greenhouse at 19–32 C.

[y] cfu = colony forming units. Values are averages of 10 replicate root samples from different pots. Population levels were determined by plating dilutions of root washes on King's medium B amended with rifampicin, cycloheximide, and benomyl.

[z] Values within a column followed by the same letter are not statistically significant at $P = 0.01$ according to Duncan's multiple range test.

average of 40% in three experiments when the plants were grown in soil infested with either *P. janthinellum* or *E. javanicum* and by 25% when grown in soils infested with *P. citreonigrum*. Root colonization by *Penicillium* spp. was significantly less on E6-treated plants grown in soils infested with these species of *Penicillium*. *P. citrinum* appeared to affect plant growth in only one of three experiments and was not significantly affected by E6. When zinnias were grown in noninfested soils, there were significant differences between the weight of water-treated and E6-inoculated plants in two of five experiments; colonization by *Penicillium* spp. was less as a result of treatment with E6 only in those experiments in which growth promotion occurred.

DISCUSSION

P. fluorescens strain E6 affected growth in a large number of species. Inoculation of seeds or cuttings with E6 resulted in increased growth of carnation, stock, sunflower, vinca, and zinnia and depressed growth of two other species. The effect of E6 varied even among different members of the same family; the growth of zinnia and sunflower within the Asteraceae were increased by inoculation with E6, whereas the growth of marigold in the same family was signficantly reduced by the treatment. A differential effect on plant species was demonstrated in a previous study by the varying responses of 10 plant species to two strains of *Bacillus subtilis* (1). It is evident from these collective findings that no single strain beneficially affects all plants and that a bacterial strain may be beneficial to one species and deleterious to another.

There was considerable variation in the results among experiments when E6 was tested for specific effects on plant growth. Typically, statistically significant results were obtained in 60–75% of the experiments. This is not surprising since the phenomenon is believed to be affected by various interacting factors, such as the composition of the soil microflora and the abiotic environment. The population sizes and the activity of particular components of the microflora undoubtedly vary among experiments with untreated soils because of cyclic changes and differences in moisture, temperature, aeration, and nutrient status. This variability is similar to that encountered when testing the pathogenicity of such pathogens as *Pythium, Fusarium*, and *Rhizoctonia* in untreated soils.

Soil type did not appear to influence the occurrence or extent of growth promotion by E6. Population densities of E6 on roots were lower in some soils, but this was not reflected in the growth response of the plant. These findings, however, may only apply to the greenhouse growing of ornamental plants where water potentials are maintained at levels ideal for E6 growth. Water potentials in the field would be expected to decrease periodically to levels which are suboptimal for the bacteria. In these situations, bacterial colonization would be severely depressed in soils with lower moisture-holding capacity, and thus, soil type could play a greater role.

This study supports the theory that an important mode of action of the PGPR in promoting plant growth is in modifying the composition of root microflora and, more specifically, in limiting the deleterious effect of certain fungal pathogens. Treatment of zinnia seed with E6 in this study resulted not only in a lower frequency of root colonization by *Penicillium* spp., but also in a higher frequency of colonization by *Fusarium* spp. It had no significant effect, however, on the total fungal or bacterial population levels. This is in contrast to the report by Kloepper and Schroth (9) that inoculation of potato seed pieces with PGPR resulted in generally decreased population densities of Gram-positive bacteria and fungi in the root zone. Suslow (19) observed both a depression in the total population density of root-colonizing fungi and a shift in the density of particular components of the fungal microflora following inoculation of sugar beet seed with PGPR.

The effect of the individual fungi associated with zinnia roots on the growth of zinnia seedlings varied markedly. Four species of *Penicillium* inhibited the growth of zinnia, whereas other fungi exhibited no effect. To examine the potential of each fungus to affect plant growth, each was introduced to soil at population densities presumably greater than that ordinarily found in untreated soils. Although this magnified any deleterious effect, it nevertheless should give an indication of their potential to influence plant growth in untreated soils. Toxin production has been suggested to be one mechanism by which *Penicillium* spp. and other minor pathogens affect plant growth (6,13). It has been reported that *P. citrinum* and *P. citreonigrum* produce the toxin citrinin in vitro and that *P. janthinellum* produces penicillic acid (17). Both toxins have phytotoxic activity (15,26).

The deleterious effects on plant growth exerted by some strains of rhizobacteria (20) and by a mixture of root-colonizing fungi, which included species of *Penicillium, Aspergillus, Alternaria*, and *Trichoderma* (19), were previously demonstrated by the inoculation of sugar beet seed with high populations of these organisms. As in this study, the application of PGPR nullified the effects of the deleterious organisms and limited their ability to colonize roots.

It is important to note that treatment with E6 can increase root colonization by specific fungi. This may be due to the direct enhancement of the activity of the particular fungi, or it may result from the elimination of competing microorganisms. In this study, species of *Fusarium* that were increased by E6 did not have a detrimental effect on the growth of zinnia. However, enhancement of pathogens, such as *Pythium* spp. and *Fusarium oxysporum*, by the application of bacteria has been documented (19,21,24).

TABLE 5. Colonization of zinnia roots by fungi as affected by preplant seed inoculation with *Pseudomonas fluorescens* strain E6

	Colonies:			
	Per 100 cm of root[x]		Percentage of total	
Fungal genus	Control	E6	Control	E6
Fusarium	17	28†[y]	11	23*
Penicillium	67	31*	39	24*
Gliocladium	6	12	3	9†
Cylindrocarpon	28	18	15	16
Other[z]	47	34	32	28
Total	165	123	100	100

[x] Average of 10 replicate root samples from different pots with 120 cm of root assayed per sample. Plants were grown in Delhi sand in the greenhouse for 3 wk at 19–32 C. Colonies were enumerated by plating roots on cellophane-extract agar for 14 days.

[y] The dagger symbol (†) and asterisk (*) indicate that the difference from the control is statistically significant according to *t*-test at $P = 0.10$ and $P = 0.05$, respectively.

[z] Includes *Alternaria, Aspergillus, Cephalosporium, Dictyosporium, Paecilomyces, Phoma, Trichoderma, Ulocladium*, and unidentified genera, each comprising less than 5% of the total fungal population.

TABLE 6. Zinnia growth in Delhi sand infested with fungi isolated from zinnia roots

Fungus[x]	Top weight[y] (g)	Difference from control (%)
Eupenicillium javanicum	1.6 a[z]	−56
Penicillium janthinellum	1.6 a	−56
P. citreonigrum	1.7 a	−53
P. citrinum	2.3 a	−36
Trichoderma sp.	2.9 ab	−19
Fusarium solani	3.5 b	−3
F. oxysporum	3.6 b	0
Cylindrocarpon sp.	3.7 b	+3
Phoma sp.	3.8 b	+6
No fungus (control)	3.6 b	...

[x] Conidia and hyphal fragments from 1-wk-old PDA cultures were added to Delhi sand at initial inoculum densities of 10^4 to 10^5 propagules per gram of soil.

[y] Mean of eight replicate pots with three plants per pot. Plants were grown for 4 wk in a greenhouse at 19–32 C.

[z] Values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 7. Growth of zinnia in soil infested with *Penicillium* spp. with and without seed treatment with *Pseudomonas fluorescens* strain E6 and colonies of *Penicillium* spp. from roots

Fungus[u]	Seed treatment[v]	Plant top wt (g)[w] in trial: 1	2	3	4	5	Colonies of *Penicillium* spp. per 100 cm of root[x] in trial: 1	2	4
Eupenicillium javanicum	Water	1.5 a[y]	1.1 a	2.7 a	···	···	81	41	···
	E6	3.5 d	2.0 bc	4.8 b	···	···	24*[z]	13*	···
Penicillium citreonigrum	Water	1.8 ab	1.5 ba	3.0 a	···	···	238	48	···
	E6	2.9 cd	2.1 bc	5.0 b	···	···	169*	12*	···
Penicillium janthinellum	Water	1.8 a	···	···	2.5 a	2.6 a	220	···	54
	E6	2.7 bcd	···	···	3.8 b	5.1 c	137*	···	22*
Penicillium citrinum	Water	2.0 abc	···	···	3.4 b	4.0 b	185	···	46
	E6	2.9 cd	···	···	3.9 b	4.5 bc	141	···	35†
No fungus control	Water	2.9 cd	1.7 b	4.2 b	3.9 b	5.2 c	7	16	20
	E6	3.0 cd	2.6 c	5.0 b	5.0 c	5.3 c	1	7†	10*

[u] Conidia from 1-wk-old PDA cultures added to Delhi sand at initial densities of 10^4 to 10^5 propagules per gram.

[v] Seeds were dipped into cell suspension of E6 (10^8 colony-forming units per milliliter) or into water.

[w] Average of eight replicate pots with three to four plants per pot. Plants were grown in a greenhouse at 19–32 C and measurements were taken 3 wk after planting.

[x] Averages of six to eight replicate root samples with approximately 120 cm of roots per sample. Roots were plated on 10% PDA containing tergitol-NPX, penicillin-D, streptomycin sulfate, and tetracycline. Colonies were enumerated after 3 days at 25 C.

[y] Values within each column followed by the same letter are not statistically significant ($P = 0.05$) according to Duncan's multiple range test.

[z] The dagger symbol (†) and asterisk (*) indicate the difference from water check in the same soil is significantly different according to Student's *t*-test at $P = 0.10$ and $P = 0.05$, respectively.

Studies of plant growth promotion and biological control that are based on greenhouse experiments are often controversial because the findings may have little applicability to field situations. The findings are meaningful, however, to the ornamental plant industry in which plants normally are grown under controlled conditions. The ability of some root-colonizing bacteria to alter the composition of the rhizosphere under these conditions suggests their potential use for protecting ornamental plants from soilborne pathogens.

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