Fig. 2. Soil pH as affected by application rate of calcium carbonate (CaCO_3) to blueberry-producing soils from eight different New Jersey sites (1 lb/acre = 1.12 $\text{kg}\cdot\text{ha}^{-1}$).

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Effects of a Microbial Inoculant on Plant Growth and Rhizosphere Bacterial Populations of Container-grown Plants

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ADDITIONAL INDEX WORDS.

Spathiphyllum, *Hibiscus rosa-sinensis*, *Dyopsis lutescens*, areca palm, fluorescent pseudomonads, actinomycetes, heat-tolerant bacteria

SUMMARY. A commercially available microbial inoculant (Plant Growth Activator Plus) that contains 50 microorganisms, primarily bacteria, was evaluated in a soilless container substrate to determine its effects on root bacterial populations and growth response of container-grown plants at three fertilizer rates. The tropical ornamental plants included hibiscus (*Hibiscus rosa-sinensis* 'Double Red'), spathiphyllum (*Spathiphyllum* 'Green Velvet') and areca palm (*Dyopsis lutescens*). The bacterial groups enumerated were fluorescent pseudomonads, actinomycetes, heat-tolerant bacteria, and total aerobic bacteria. Analysis of the inoculant before its use determined that fluorescent pseudomonads claimed to be in the inoculant were not viable. The plant variables measured were plant color rating, shoot dry weight and root dry weight. Only hibiscus shoot dry weight and color rating increased in response to the addition of the inoculant to the substrate. Hibiscus roots also had a significant increase in the populations of fluores-

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cent pseudomonads and heat-tolerant bacteria. From a commercial production point of view, increasing fertilizer rates in the substrate provided a stronger response in hibiscus than did addition of the microbial inoculant. Furthermore, use of the inoculant in this substrate did not compensate for reduced fertilizer inputs.

An increasing number of microbial-based products claim to increase plant growth or protect plants from various pests. Products that claim to directly control plant pests are referred to as biological pesticides or biopesticides. These products are regulated by the Biopesticides and Pollution Prevention Division of the U.S. Environmental Protection Agency (EPA). There are three types of biopesticides—biochemical, plant, and microbial. The latter contain a naturally occurring or genetically altered microorganism or its product as the active ingredient (U.S. Environmental Protection Agency, 2001).

If a microbial product only claims to improve plant health in general, without mentioning direct control of specific pests, the product does not have to be registered by EPA. This group of products is often referred to as inoculants. Root-associated (rhizosphere) bacteria that benefit plant growth are called plant-growth-promoting rhizobacteria (PGPR). However, that term can be misleading, as pointed out by Lazarovits and Nowak (1997). For example, PGPR that promote the growth of one species may be detrimental to another species, or the PGPR are only beneficial to plants under specific environmental situations, such as high disease pressure or low nutrient levels.

Some products contain only a single microorganism, whereas others contain a mixture of microorganisms. The latter approach is probably more useful, because at least one of the microbes in the mixture may benefit the targeted host plant. Many of these microbial products claim to reduce fertilizer use because they include bacteria that fix nitrogen nonsymbiotically or liberate phosphates and micronutrients from the potting substrate. As with chemical products, microbial products are formulated with various carrier compounds, both inorganic and organic. Carrier compounds may include very small amounts of plant nu-

trients (nitrogen, phosphorous, potassium), sugars, amino acids, plant hormones, etc., compounds that may also affect plant growth. Because evaluation of microbial inoculant products normally includes only plant growth responses, without examining the microbial responses, it is difficult to determine if the plant responses observed may be due to the microbes added to the plant-growth substrate.

The experiment described herein was conducted on container-grown tropical ornamental plants, which are typically grown in substrates that are not pasteurized (e.g., steam sterilized or fumigated with a chemical). Common substrate components include pine bark, coir, sand, peat, perlite, and vermiculite, materials that naturally contain microbes. The objectives of this research were to determine the effects of a commercially available microbial inoculant on root bacterial populations and growth response of plants at three fertilizer rates, using standard industry practices.

Materials and methods

FERTILIZER AND BIOLOGICAL GROWTH ACTIVATOR TREATMENTS.

Two- to three-leaf areca palm seedlings, tissue-cultured spathiphyllum plugs, and rooted hibiscus cuttings were planted on 10 Feb. 2000 into 2.8-L (1-gal) plastic containers filled with a substrate composed of 5 pine bark, 4 sedge peat, and 1 sand (by volume). The substrate was amended with Micromax (Scotts Co., Marysville, Ohio) at $890 \text{ g}\cdot\text{m}^{-3}$ ($1.5 \text{ lb}/\text{yard}^3$) and dolomitic limestone at $7.1 \text{ kg}\cdot\text{m}^{-3}$ ($12 \text{ lb}/\text{yard}^3$). Osmocote 15-9-12 [15N-4P-10K; 8 to 9 month formulation at 21°C (70°F); Scotts Co.] was incorporated into the substrate before planting at three rates of $2.02 \text{ kg}\cdot\text{m}^{-3}$ ($3.41 \text{ lb}/\text{yard}^3$), $4.05 \text{ kg}\cdot\text{m}^{-3}$ ($6.82 \text{ lb}/\text{yard}^3$), or $8.09 \text{ kg}\cdot\text{m}^{-3}$ ($13.6 \text{ lb}/\text{yard}^3$). These rates represented 25%, 50%, and 100% of the manufacturer's recommended rate, respectively. The lower fertilizer rates were included to evaluate the claim that use of the microbial inoculant can reduce the amount of fertilizer necessary to produce a quality crop. For each fertilizer rate, 10 replicate containers per plant species had Plant Growth Activator Plus (PGA Plus) (Organica, Inc., Kings Park, N.Y.) incorporated into the substrate at the recommended rate of $297 \text{ g}\cdot\text{m}^{-3}$ ($0.5 \text{ lb}/\text{yard}^3$). Ten additional containers

per fertilizer treatment had no PGA Plus incorporated into the substrate.

Areca palms and hibiscus were grown in a shadehouse covered with 55% light exclusion shade cloth [maximum photosynthetic photon flux (PPF) = $855 \text{ mE}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$], while the spathiphyllums were grown under 73% light exclusion (maximum PPF = $513 \text{ mE}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). All plants received about 2 cm (0.75 inch) of water daily from overhead irrigation, plus natural rainfall. The hibiscus were harvested on 10 May 2000, and the spathiphyllums and areca palms were harvested on 24 and 28 Aug. 2000, respectively. On those dates, plant color was rated subjectively on a scale of 1 to 10, with 10 = darkest green, 5 = light green, and 1 = completely yellow. Plant shoots were excised at the soil line and the roots were rinsed free of substrate. A sample of the root system from four replicate plants per treatment and species were used to assay microbial populations, as described below. Roots and shoots were oven-dried at 62.7°C (145°F) until constant weight was achieved.

BACTERIAL ENUMERATION ASSAYS.

Assay procedures were based on published techniques (Elliott and Des Jardin, 1999). Because the label indicated that the PGA Plus contained pseudomonads *Bacillus* and *Streptomyces*, these bacterial groups plus total aerobic bacteria were enumerated throughout the study using the following media: S1 medium for fluorescent pseudomonads (Gould et al., 1985); humic acid vitamin agar (HAVA) for actinomycetes, including *Streptomyces* sp. (Hayakawa and Nonomura, 1987); solidified 10% Bacto tryptic soy broth (10% TSBA), amended with $100 \mu\text{g}\cdot\text{mL}^{-1}$ (ppm) cycloheximide, for heat-tolerant bacteria and total aerobic bacteria. The heat-tolerant bacteria were enumerated by placing the dilutions in an 80°C (176°F) water bath for 10 min before spreading on the 10% TSBA. All inoculated media were incubated at 28°C (82°F). Fluorescent pseudomonads were counted after 24 and 48 h incubation using a shortwave UV lamp. Counts of total and heat-tolerant bacteria were made at 7 d, and actinomycetes at 14 d.

Before the experiment was initiated, four samples of PGA Plus were obtained from the container to determine the number of culturable bacteria in the material. Each 5-g (0.18-oz) sample was placed into a 250-mL flask with 45 mL of sodium pyrophosphate

Table 1. Bacterial numbers associated with a microbial inoculant product, Plant Growth Activator Plus (PGA Plus), plant-growth substrate, and roots of hibiscus or areca palm grown with or without the addition of PGA Plus.

Bacterial group	PGA Plus ^y	Substrate ^y	Log ₁₀ colony forming unit/g dry weight ^z			
			Hibiscus roots ^x		Areca palm roots ^x	
			No PGA Plus	PGA Plus	No PGA Plus	PGA Plus
Fluorescent pseudomonads	0	3.63	5.68*	6.07	5.14	5.19
Actinomycetes	6.12	6.22	6.82	6.76	6.32	6.38
Heat-tolerant bacteria	6.32	5.94	6.03*	6.28	6.17	6.17
Total bacteria	6.56	7.36	8.54	8.52	8.18	8.29

^z28.35 g = 1.0 oz.^yValues are means of four (PGA Plus) or eight (substrate) samples obtained just before planting.^xValues are means of four replicate plant root systems at termination of the experiment, 3 months for hibiscus and 6 months for areca palm. Bacterial population value pairs, within each plant species, followed by an asterisk are significantly different at $P = 0.05$, according to LSD t test.

buffer with glycerol (buffer) (Elliott and Des Jardin, 1999). The flask was placed on a rotary shaker (200 rpm) for 20 min. A 10-fold dilution series was then conducted. Aliquots (0.1 mL) were spread on the media described above. Also before the experiment was initiated, eight samples of the substrate were obtained to determine the number of culturable bacteria in it. Each 10-g (0.35-oz) wet weight sample was placed into a 250-mL flask with 90 mL of buffer. The flask was shaken, a dilution series conducted and 0.1-mL aliquots spread on the media described previously. The substrate material was then collected by filtration onto a dried, preweighed filter paper, and dried at 62.7 °C until a constant weight was obtained.

For each of the randomly selected plant replications (four for each plant species), 10 g of wet weight root material was placed into a 250-mL flask with 100 mL of buffer (Elliott and Des Jardin, 1999). The flask was shaken, a dilution series completed, and 0.1-mL aliquots spread on the previously described media. Root materials were then collected

by filtration onto a dried, preweighed filter paper, and then dried at 62.7 °C until a constant weight was obtained.

STATISTICAL ANALYSIS. Plant quality data and rhizosphere microbial data were analyzed by analysis of covariance, with fertilizer rate as the continuous variable and PGA Plus treatment as the qualitative variable using PROC GLM (SAS Institute, Cary, N.C.). Due to laboratory technical problems, bacterial enumeration data from spathiphyllum roots were not evaluated.

Results and discussion

The PGA Plus label indicated that it is a proprietary blend of various species of the bacterial genera *Bacillus*, *Streptomyces*, and various beneficial *Pseudomonas* at concentrations of 1.1×10^9 colony-forming units (CFU)/g (5×10^{11} CFU/lb) of material, with a shelf life of 2 years. The label also indicated it contained *Trichoderma* (a fungus), phosphorus-solubilizing bacteria, nitrogen-fixing bacteria, cytokinin-producing bacteria, essential amino acids, various vitamins, and natural sugars. The PGA

Plus provided for our experiments contained no viable fluorescent pseudomonads, a group of bacteria normally considered beneficial to plants (Table 1). The PGA Plus was received in December 1999 and stored at room temperature until it was sampled in February 2000, well within the 2-year shelf life. However, the lack of viable fluorescent pseudomonads is not surprising. As observed with seed treatment studies, gram-negative bacteria, such as *Pseudomonas* and related genera, are not considered to have a long shelf life (Elliott et al., 2001; Emmert and Handelsman, 1999; Mathre et al., 1999). The lack of conformity with a label is not unprecedented, even with EPA-registered microbial products (Elliott et al., 2001).

Both *Streptomyces*, a genus of actinomycetes, and heat-tolerant bacteria, which include *Bacillus* sp., were present in the PGA Plus (Table 1). All four enumerated bacterial groups were also present in the plant-growth substrate (Table 1). The latter was not unexpected as the substrate was not pasteur-

Table 2. Rhizosphere bacterial populations associated with hibiscus and areca palms, grown with or without the microbial inoculant Plant Growth Activator Plus (PGA Plus) at three fertilization rates. Data are means from four replicate plants $\pm$ SE.

Microbial inoculant treatment	Fertilizer rate (g/pot)	Log ₁₀ colony forming units per gram dry weight of root ^z							
		Hibiscus				Areca palm			
		Fluorescent pseudomonads	Actinomycetes	Heat-tolerant bacteria	Total bacteria	Fluorescent pseudomonads	Actinomycetes	Heat-tolerant bacteria	Total bacteria
No PGA Plus	5	5.97 $\pm$ 0.24	6.63 $\pm$ 0.12	6.34 $\pm$ 0.07	8.47 $\pm$ 0.05	4.63 $\pm$ 0.32	6.33 $\pm$ 0.22	6.18 $\pm$ 0.08	8.14 $\pm$ 0.05
	10	5.36 $\pm$ 0.25	6.93 $\pm$ 0.11	5.94 $\pm$ 0.06	8.55 $\pm$ 0.21	5.41 $\pm$ 0.21	6.18 $\pm$ 0.08	6.24 $\pm$ 0.06	8.22 $\pm$ 0.12
	20	5.72 $\pm$ 0.28	6.91 $\pm$ 0.19	5.91 $\pm$ 0.06	8.60 $\pm$ 0.13	5.38 $\pm$ 0.28	6.44 $\pm$ 0.18	6.09 $\pm$ 0.04	8.50 $\pm$ 0.03
PGA Plus	5	6.14 $\pm$ 0.11	6.87 $\pm$ 0.03	6.37 $\pm$ 0.03	8.46 $\pm$ 0.06	5.14 $\pm$ 0.07	6.17 $\pm$ 0.24	6.18 $\pm$ 0.06	8.15 $\pm$ 0.03
	10	5.99 $\pm$ 0.13	6.65 $\pm$ 0.18	6.12 $\pm$ 0.03	8.47 $\pm$ 0.05	5.29 $\pm$ 0.05	6.43 $\pm$ 0.22	6.13 $\pm$ 0.07	8.08 $\pm$ 0.13
	20	6.09 $\pm$ 0.15	6.76 $\pm$ 0.12	6.35 $\pm$ 0.17	8.63 $\pm$ 0.08	5.12 $\pm$ 0.08	6.54 $\pm$ 0.18	6.21 $\pm$ 0.09	8.32 $\pm$ 0.06
Statistical significance (P)									
PGA Plus		0.0325	NS	0.0070	NS	NS	NS	NS	NS
Fertilizer rate		NS	NS	0.0063	NS	NS	NS	NS	0.0062
PGA Plus $\times$ fertilizer		NS	NS	NS	NS	NS	NS	NS	NS

^z28.35 g = 1.0 oz.

Table 3. Plant color ratings and root and shoot dry weights for spathiphyllum, hibiscus, and areca palms, grown with or without the microbial inoculant Plant Growth Activator Plus (PGA Plus) at three fertilization rates. Data are means from 10 replicate plants $\pm$ SE.

Microbial inoculant treatment	Fertilizer rate (g/pot) ^z	Spathiphyllum			Hibiscus			Areca palm		
		Root dry wt (g)	Shoot dry wt (g)	Color ^y	Root dry wt (g)	Shoot dry wt (g)	Color	Root dry wt (g)	Shoot dry wt (g)	Color
No PGA Plus	5	11.2 $\pm$ 1.1	11.5 $\pm$ 1.8	6.2 $\pm$ 0.1	5.9 $\pm$ 0.8	16.5 $\pm$ 5.9	8.3 $\pm$ 0.1	6.3 $\pm$ 0.9	12.6 $\pm$ 1.4	8.8 $\pm$ 0.2
	10	14.8 $\pm$ 1.1	18.0 $\pm$ 2.1	7.0 $\pm$ 0.2	6.0 $\pm$ 0.5	20.1 $\pm$ 1.6	9.4 $\pm$ 0.1	6.3 $\pm$ 0.8	15.3 $\pm$ 1.8	9.3 $\pm$ 0.1
	20	13.3 $\pm$ 2.8	24.6 $\pm$ 2.7	8.7 $\pm$ 0.2	7.4 $\pm$ 0.4	26.4 $\pm$ 1.2	9.8 $\pm$ 0.1	4.6 $\pm$ 0.6	16.1 $\pm$ 1.9	9.6 $\pm$ 0.1
PGA Plus	5	9.2 $\pm$ 1.5	8.8 $\pm$ 1.3	6.2 $\pm$ 0.1	7.2 $\pm$ 0.6	19.6 $\pm$ 1.3	8.2 $\pm$ 0.1	6.2 $\pm$ 0.3	12.7 $\pm$ 0.8	8.8 $\pm$ 0.1
	10	11.0 $\pm$ 1.3	16.3 $\pm$ 2.1	7.1 $\pm$ 0.2	7.5 $\pm$ 0.7	24.5 $\pm$ 2.0	9.0 $\pm$ 0.1	6.0 $\pm$ 0.8	14.4 $\pm$ 1.9	9.5 $\pm$ 0.1
	20	12.7 $\pm$ 1.3	22.3 $\pm$ 2.4	8.8 $\pm$ 0.2	7.4 $\pm$ 0.9	26.9 $\pm$ 1.5	9.8 $\pm$ 0.1	4.7 $\pm$ 0.5	12.8 $\pm$ 1.5	9.7 $\pm$ 0.1
Statistical significance (<i>P</i>)										
PGA Plus		NS	NS	NS	NS	0.042	0.039	NS	NS	NS
Fertilizer rate		NS	0.0001	0.0001	NS	0.0001	0.0001	0.025	NS	0.0001
PGA Plus $\times$ fertilizer		NS	NS	NS	NS	NS	NS	NS	NS	NS

^z28.35 g = 1.0 oz.

^y10 = darkest green, 5 = light green, 1 = completely yellow.

ized, conforming with standard practices in the industry. This means that, for example, about 8.3×10^8 CFU of total bacteria from PGA Plus was added to 1 yard³ of substrate that already contained about 5.3×10^{12} CFU of total bacteria.

For hibiscus roots, across all fertilizer rates, significant increases ($P < 0.05$) in the fluorescent pseudomonad and heat-tolerant bacterial populations were observed for PGA Plus, but not in the actinomycete or total bacterial populations (Tables 1 and 2). Because there were no viable fluorescent pseudomonads in the PGA Plus, the increase observed cannot be attributed directly to the addition of fluorescent pseudomonads. The increase may be due a component of the PGA Plus that affected fluorescent pseudomonad populations already present in the substrate or to root exudates. There were no population differences for any of the bacterial groups enumerated on the areca palm roots (Tables 1 and 2).

For hibiscus roots, the lowest fertilizer rate resulted in a higher population of heat-tolerant bacteria (Table 2). For areca palm roots, the highest fertilizer rate increased the population of total aerobic bacteria (Table 2). There were no significant interactions between PGA Plus and fertilizer rate (Table 2).

Addition of the PGA Plus to the substrate used to grow the three plant species resulted in significant increases in hibiscus shoot dry weight and color rating, but had no effect on the other two species (Table 3). However, fertilizer treatments accounted for a greater percentage of variation (85%) than the PGA Plus only treatments (13%) for

hibiscus shoot dry weight and color rating. Increased fertilizer rates increased shoot dry weight of all species but was only significant for spathiphyllum and hibiscus. Increased fertilizer rates significantly increased the color rating for all species. Root dry weight for areca palms was significantly decreased by increasing fertilizer rates. No significant interactions between PGA Plus and fertilizer rates were found. Thus, the claim that PGA Plus reduces fertilizer requirements (Organica, Inc., personal communication) is not supported by these data. It is interesting that only hibiscus, a woody dicot, responded positively to this product and the two monocots, spathiphyllum and areca palms, did not. The reasons for this species-specific response are not known.

Conclusions

Shoot dry weight and color rating of hibiscus increased when the PGA Plus microbial inoculant was incorporated into the substrate, as did populations of fluorescent pseudomonads and heat-tolerant bacteria. However, fluorescent pseudomonads were not present as viable bacteria in the PGA Plus; this bacterial group was only present in the substrate. It is possible that the other components in the PGA Plus influenced this bacterial population. Alternatively, hibiscus root exudates may be the influential factor. From a commercial production point of view, increasing fertilizer rates provided a stronger positive plant growth and quality response in hibiscus than did the addition of the microbial inoculant. Furthermore, use of the PGA Plus in this substrate did not compensate for reduced fertilizer inputs.

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