Microbial Soil Amendments Do Little to Improve Citrus Tree Performance in Florida Soils

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The profitability of Florida citrus groves can be limited by poor soil conditions, including low nutrient and low waterholding capacity. There are many advertised soil amendments that claim to "condition" unproductive soils by improving the balance of beneficial microbes even though there is no known research demonstrating the effectiveness and profitability of these products on citrus. We tested four commercially available microbial-based liquid soil amendment products that have been recommended by their manufacturers to be beneficial for citrus. In three repeated greenhouse studies, products were applied at recommended and higher rates to seedlings of Carrizo citrange grown in pots of native Candler sandy soil. In Experiment I, total plant growth tended to increase in response to amendments of two of the products (B, C), but growth responses to increased rates of B and C were not conclusive. Nitrogen leaching and leaf N responses were not remarkable. The other two products (A, D) had no effect on seedling growth or N budgets. Greenhouse experiments were repeated using B and C at high or low nutrition, but seedling growth and mineral nutrient status were little affected. Nonetheless, products B and C were tested in three field-scale experiments at three locations (Southern flatwoods, Indian River, and central Ridge). The two products were applied at recommended rates with or without biosolids (sludge) to provide additional soil organic matter (SOM) microbial substrate. During 3 years of repeated applications and monitoring, the microbial products did not consistently affect any measured parameter in the soil or citrus crop. Soil measurements included SOM, pH, CEC, P, K, Ca, Mg, Fe, Cu, microbial respiration, PWP, and soil *Phytophthora* **populations. Leaf analyses included color (SPAD), N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B. Tree canopy heights and volumes were measured, and fruit quality was expressed as fruit size, percentage juice, brix, acid, and ratio. Fruit yield could not be rigorously assessed because in 2 years the harvest was damaged by hurricanes, and one experiment was destroyed due to the canker eradication rule in Florida. Based on greenhouse and field studies, these microbial-based liquid soil amendment products were of little or no benefit. The additional cost and labor of incorporating these products into Florida citrus production programs could not be justified.**

Citrus in Florida is grown on sandy soils with low water and nutrient holding capacities and little organic matter. Organic soil amendments to the surface or incorporated into the soil may increase soil nutrient holding capacity and thereby improve yields and profits. Research studies on Florida citrus indicate that application of organic compost increases available soil moisture (Muchovej et al., 2006). Biosolids and chicken manure have also been used successfully in citrus production, but several constraints such as phosphorus loading restrict the frequent use of these materials. As an alternative to bulky organic soil amendments, many products with concentrated microbial formulations advertise to improve soil characteristics, reduce tree water stress, and improve plant performance. Growers have reported that microbial products reduced tree water stress. Most Florida citrus soils could benefit from improved soil characteristics, but research is required to determine if soil amendments of microbes and/or bio-solids will work as advertised and actually improve profits in Florida citrus production. These amendments could be of particular value in the management of sand soaks or other excessively leached areas.

In a recent study conducted in Florida, a soil microbial product ("Equity") did not enhance any measurable growth parameter, including tuber yield, overall tuber quality, or early plant vigor of 'Atlantic' potato at either full fertilizer rates or at a 25% reduced rate (Hutchinson, 2003). Only the fertilizer rate itself produced a significant response on the potato crop. There are no data on the efficacy of microbial soil amendments on Florida citrus. We therefore tested the effects of four commercially available microbial soil conditioners for Florida citrus. If these products benefit Florida citrus soils, they should improve growth and nutrient content of seedlings grown in a representative sandy Ridge soil in the greenhouse. In addition, we tested two products in the field to determine if tree growth and yields respond to microbial amendments with or without biosolid additions in three major growing areas. We also hypothesized that effective microbial products applied in the field would improve chemical and physical soil properties.

Materials and Methods

GREENHOUSE EXPERIMENT 1. In the first greenhouse experiment conducted in 2003, three rates of each of four products [A $=$ Equity (Naturize BioSciences LLC, Jacksonville, FL); B $=$ HMS1+2 (Helena Chemical Co., Collierville, TN); C = Superbio (Advanced Microbial Solutions, Pilot Point, TX); D = Biozone WP+liquid (International Biotech Inc., Mulberry, FL)] were evaluated using well-fertilized 8-month-old Carrizo citrange seedlings growing in 150-mL conetainers filled with unsterilized Candler

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fine sand (hyperthermic, coated Typic Quartzipsamments) from Polk County, FL. All products were applied twice, once at the beginning and again after 60 d, at the recommended rate (2 qt/acre $= 1x$), and at 2x and 4x rates along with an unamended control. The products were applied with water at the equivalent rate of 10 gal/acre. The amounts of product and water dispensed per seedling were calculated according to the soil surface area of the conetainers. Treatments were replicated using seven replicate seedlings. Two randomly selected pots from each treatment were weighed every second day, and their weight loss was used to estimate total water use by evapotranspiration (ET). The average weight loss from each treatment was replaced with a dilute complete nutrient solution $(8-2-8)$ with an N concentration of 200 mg·L⁻¹. Nutrient leaching from the pots was avoided and each seedling received about 5 mg N/week/pot. Once every 2 weeks, each pot was leached with about 40 mL of water and the leachate was analyzed for total N. The leachate from five replicate blank pots with no plant that received the 2x treatment of the four products was also collected and analyzed to determine soil N losses without interference from roots. Total N applied, leached, and N taken up as calculated by the difference corrected N lost by the no plant treatment was used to develop plant/soil N budgets for each treatment. Seedling growth and leaf N levels were evaluated after 90 d.

GREENHOUSE EXPERIMENT 2. Experiment 2 was conducted in 2005 with two microbial products (B, C) selected from Experiment 1 based on seedling growth performance. Three rates of each product were applied to six replicate seedlings the same way as in the first greenhouse experiment. Leachate data were not collected, but seedling growth and leaf N levels were evaluated. The seedlings were watered enough to leach 20 to 30 mL every 2 to 3 d using a dilute complete nutrient solution (8–2–8) diluted to 100 mg \cdot L⁻¹ of N.

GREENHOUSE EXPERIMENT 3. In 2007, the procedure of Experiment 2 was repeated except the seedlings received water only with no fertilizer for the first 7 weeks after transplanting to reduce their nutrient status. Uniform seedlings were randomly divided into seven treatments: three rates of products B and C and an untreated control, each with seven replicate seedlings. Twice per week (Monday, Wednesday) plants were watered to soil saturation avoiding leaching, and once per week (Friday) the seedlings were fertilized at a low rate with 20 mL of 8–2–8 diluted to 150 mg·L⁻¹ of N. Thus, each seedling received about 3 mg N/week/plant. After 8 weeks, a second application of microbial amendments was repeated at the same rates. The plants were harvested after a total of 14 weeks when seedling growth and leaf N levels were evaluated.

FIELD EXPERIMENTS. Three large replicated field experiments, using plot sizes from 1.0 to 2.5 acres, were established on the Ridge (young 'Hamlin' orange, near Ft. Meade), Indian River (mature 'Valencia' orange, near Ft. Pierce) or Southern Flatwoods (mature 'Valencia' orange, near Basinger) citrus growing areas. The two best microbial products (B, C) were selected from the greenhouse screening experiments during Winter 2003–04 for additional testing in the field. The first season was used to establish baseline yield, leaf nutrients, and soil properties. The two microbial product treatments were applied in the spring of 2004 at recommended rates (0.5 gal in 50 gal of water/acre) using a herbicide boom sprayer along with an untreated control and repeated after 30 d as recommended by the manufacturers. Each microbial product treatment was combined with or without a 1000 lb/acre application of bio-solids to form a full factorial treatment combination of 2 products and a control \times 2 levels of biosolids with four replications in a randomized complete-block design. The biosolids were recommended by some manufacturers to provide microbial substrate. Standard grove management practices were maintained in the experiments for the duration of the study. All experiment treatments were repeated at the three sites in the spring of 2005 and 2006.

Soil samples were collected from the 0–15 cm depth in the fall of each year for detailed analysis. Soil measurements included soil organic matter (SOM; 500 °C loss on ignition method), pH $(1:2.5$ diluted in H₂O), cation exchange capacity (CEC; pH buffer method), extractable P, K, Ca, Mg, Fe, Cu (Mehlich-1 method), microbial respiration $(CO₂$ evolution method), gravimetric water content, water content at the permanent wilting point water potential (PWP; chilled mirror dewpoint method), bulk density (volumetric scoop method), and counts of *Phytophthora* colony forming units. Leaf samples of non-fruiting spring flush leaves were collected in July each year. The leaf chlorophyll index was determined on fresh leaves using the SPAD-502 meter (Konica Minolta, Ramsey, NJ; Jifon et al., 2005). The leaves were then dried to constant mass at 70 °C and ground to a powder with a Cyclotec mill (FOSS NIRSystems, Inc., Laurel, MD) before analysis of mineral nutrient concentrations (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B) using standard methods (Waters Agricultural Laboratories, Camilla, GA).

Tree canopy heights and volumes in the plots were measured annually using an automated ultrasonic array (Schumann and Zaman, 2005). Fruit yield was estimated either from georeferenced fruit harvesting tub positions or a photographic method (Schumann et al., 2007); and fruit quality was expressed as fruit size, percentage juice, brix, acid, and ratio, using standard processing plant analytical procedures.

DATA ANALYSIS. Plant growth and nutrient status data were analyzed using analysis of variance (ANOVA) and the F-test to determine statistical significance of treatment differences (Genstat 5.0, Lawes Agricultural Trust, Rothamsted, UK). Analysis of covariance (ANCOVA) was used for yield analysis of field experiments which accounted for the original variability of tree size in the grove. Separation of significantly different treatment means was achieved with Duncan's multiple range test (DMRT) at $P < 0.05$, and linear regression analysis was used to test microbial amendment rate effects on plant growth (SAS Institute Inc., Cary, NC).

Results and Discussion

GREENHOUSE EXPERIMENT 1. The blank pots without plants required less water so they received less N than the other treatments (Table 1). Product A increased N leaching above that from the non-amended control plants so as to reduce calculated N uptake regardless of the amendment rate. Product A also tended to reduce soil pH. Product B decreased N uptake at the 1x rate but had little consistent effect on soil N budgets at the higher rates. Product C reduced the percentage of N uptake at the 1x rate but the 2x and 4x rates did not affect % N uptake. Product D increased the percentage of N leached but not significantly. Products B, C, and D had no consistent effects on soil pH.

Products B and C tended to increase the leaf dry weight (DW) and significantly increased total plant DW (TPDW) by about 50% to 60%, whereas two products (A and D) had no effect on plant growth (Table 2). However, there was no growth difference between the 2x and 4x rates of product B, and the 4x rate of product C did not grow more than the non-amended control

Table 1. Greenhouse Expt. 1. Effect of concentration (x) of four soil amendment products $(A-D)$ on average $(n = 5)$ total N applied, leached, percentage leached, calculated N uptake, percentage N taken up and soil pH of four soil amendment products applied at recommended (1x), 2x, and 4x rates to 1-year-old Carrizo citrange seedlings compared to the un-amended control treatment. N leaching was also collected from a blank treatment with no plant (NP) that received the 2x treatment of each of the four products.

					Blank	Blank	
		${\bf N}$	N	$\mathbf N$	corrected	corrected	
		applied	leached	leached	N uptake	N uptake	Soil
TMT	$\mathbf n$	(mg)	(mg)	$(\%)$	(mg)	$(\%)$	pH
$NP_A 2x$	5	$\overline{75}$	49.5 az	66.1 a	$---y$	$---$	$---$
Plant control	5	113	42.2 _b	37.2 c	49.8 a	43.9 a	3.96a
A _{1x}	5	105	53.4 a	50.8 _b	26.4 _b	25.1 _b	3.74 ab
A 2x	5	104	51.1 a	49.3 _b	27.1 _b	26.2 _b	3.67 _b
A 4x	5	107	49.9 a	46.6 _b	31.7 _b	29.6 _b	3.55 b
$NP_B 2x$	5	75	49.6 a	66.0 a			$---$
Plant control	5	113	42.2 b	37.2c	49.8 b	43.9 a	3.96 ab
B_1x	5	118	52.2 a	44.0 b	40.7c	34.3 b	3.74 _b
B 2x	5	120	46.0 ab	38.3 c	48.6 b	40.4 ab	3.89 ab
B 4x	5	133	47.0 ab	35.2c	60.8a	45.6a	4.01a
$NP_C 2x$	5	73	49.7 NS	67.6a			
Plant control	5	113	42.2	37.2 bc	49.8 c	43.9 a	3.96 ab
C _{1x}	5	98	42.0	42.7 _b	32.5d	33.1 _b	4.19a
C _{2x}	5	141	45.4	32.1c	72.2 a	51.0 a	4.05 ab
C _{4x}	5	128	44.4	34.8 c	59.4 b	46.5a	3.79 _b
$NP_D 2x$	5	73	53.1 a	72.9 a	---		
Plant control	5	113	42.2c	37.2 _b	49.8 NS	43.9 NS	3.96 ab
D _{1x}	5	120	52.3 ab	43.5 _b	48.1	40.1	3.80 _b
D 2x	5	114	43.8 bc	38.5 _b	50.3	44.2	3.86 ab
D 4x	5	102	40.7c	40.0 _b	41.3	40.6	4.15 a

zValues within a product treatment followed by different letters differ significantly (DMRT) at $P < 0.05$; NS = nonsignificantly different.

yNot determined.

Table 2. Greenhouse Expt. 1. Effect of concentration (x) of four soil amendment products (A–D) on average plant growth parameters and leaf N of n replicate seedlings of 1-year-old Carrizo citrange.

Values are means of n plants. Values followed by different letters differ significantly (DMRT) at *P* < 0.05.

plants (Fig. 1). Such a non-linear response makes drawing strong conclusions about growth effects difficult. Thus, products B and C significantly increased plant growth as estimated from TPDW while products A and D did not affect growth. Nonetheless, on this basis, products B and C were selected for further studies in the field. All four amendments reduced the root/shoot (Rt/St) DW ratio (Table 2) implying that root efficiency was increased

as fewer roots could support larger shoots. All leaf N levels were relatively high $(>3\%$ DW) and only Product C at the 2x rate increased leaf N above that of the non-amended control plants. Expressing leaf N on a leaf area generally followed the same pattern as leaf N on a dry weight basis supported by the observation that the treatment effects on leaf dry weight per area were small and not consistent.

Fig. 1. Greenhouse Expt. 1. Effect of concentration (x) of four soil amendment products (A–D) on average total plant dry weight (TPDW; $n = 8$ to 10 ± 1 SE) of 1-year-old Carrizo citrange seedlings. Coefficients of determination (R^2) and significance level of linear regressions appear in each panel; $\text{NS} = \text{nonsignificant}$.

GREENHOUSE EXPERIMENT 2. There were no treatment effects on total leaf DW or total plant growth (Table 3). Although product B at the 2x rate tended to reduce root growth, this effect was not significant. There were no significant treatment effects on Rt/St ratio. Although leaf N concentrations were uniformly high $(>=2.9\%)$, product C increased leaf N at the 1x rate when expressed on a DW basis but not at the higher rates. This effect disappeared when leaf N was expressed on a leaf area basis, implying that there may have been treatment effects on leaf tissue density or leaf DW per area.

GREENHOUSE EXPERIMENT 3.There were no significant product B and C treatment effects on leaf DW, root growth, total plant growth, or Rt/St ratio (Table 4). Thus, there was no effect of either product on plant growth in the greenhouse. Overall, leaf N was adequate but was lower in this experiment (2.4% to 2.7% DW) than in Expt. 1 and 2. Leaf N, however, was significantly reduced by the high rates of both products. We also evaluated the effects of these two products on single leaf photosynthesis (net assimilation of $CO₂$) and stomatal conductance using a portable photosynthesis system (LI6200, Li-Cor, Lincoln, NE). The small

treatment effects on leaf N apparently were not great enough to affect leaf gas exchange as there were no treatment effects on leaf photosynthesis or water use efficiency (data not shown).

FIELD EXPERIMENTS. The field experiments suffered a series of disease and hurricane problems, and consequently, the fruit yield data presented here are incomplete. The Southern Flatwoods experiment near Basinger was destroyed after the first year due to the citrus canker disease eradication rule in Florida. The remaining two experiments suffered serious tree damage from three hurricanes which passed through central Florida in the late summer of 2004. The Indian River experiment was damaged again in 2005 from Hurricane Wilma. As a result, the best fruit yield data was obtained for 2 consecutive years from the young 'Hamlin' experiment on the Ridge. Fruit samples were collected from the Indian River 'Valencia' trees for analysis of quality parameters only. Data for years 2 and 3 of the field experiments are presented in Tables 5 to 8. The first year was considered baseline data and was used in analysis of covariance (ANCOVA) where appropriate. Due to the

large amount of data collected, only statistically significant results $(P < 0.05)$ are presented in Tables 5–8.

In general, very few statistically significant treatment responses could be detected in the field experiments (Tables 5–8). However, where known responses such as increased leaf P or Fe with biosolids amendments were anticipated, they were also detected in the measured variables (Table 5). That observation confirmed the validity of the experimental design and assured our confidence in the accuracy of measured variables. Application of biosolids to these soils significantly reduced soil pH due to nitrification of ammonium-N (Table 5). Soil P was significantly increased by biosolids in the Indian River experiment, and extractable soil Fe was increased in both field sites and both years by biosolids (Table 5). Biosolids contain large amounts of Fe residues added during the sewage treatment process. Soil Ca and CEC were reduced by biosolids in 2005 at the Indian River site, possibly due to some complexation or adsorption of soil cations with the biosolids (Table 5). The same reduction of extractable soil K was observed in the Indian River site in 2006. The two microbial products B and C had no measurable effect on any of

Table 3. Greenhouse Expt. 2. Effect of concentration (x) of two soil amendment products (B, C) on average plant growth parameters and leaf N of six replicate seedlings of 1-year-old Carrizo citrange.

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	Plant	Leaf DW	Root DW	TPDW	Rt/St	Leaf N	Leaf N
Treatment	n	$\left(\mathbf{g}\right)$	(g)	(g)	dr wt ratio	$(\%)$	(mmol/m ²)
Control	12	1.05 _{NS}	1.45 ab	3.97 NS	0.58 ab	2.99 _b	137 NS
B _{1x}	6	1.10	1.44 ab	4.13	0.54 _b	3.13 ab	144
2x	6	1.05	1.34 _b	3.76	0.56 ab	3.09 ab	141
4x	6	0.97	1.49a	3.88	0.63a	3.15 ab	143
C _{1x}	6	1.06	1.38ab	3.90	0.57 ab	3.20a	163
2x	6	1.09	1.48 ab	4.12	0.56 ab	3.08 ab	145
4x	6	1.10	1.42 ab	4.08	0.54 _b	3.10ab	148

Values followed by different letters differ significantly (DMRT) at $P < 0.05$; $\text{NS} = \text{nonsignificantly different}}$.

Values followed by different letters differ significantly (DMRT) at $P < 0.05$; NS = nonsignificantly different.

Table 5. Soil analyses of two field experiments during 2 years of treatment with microbial soil conditioners.

Table 6. Leaf nutrient analyses of two field experiments during 2 years of treatment with microbial soil conditioners.

NS, *, **, ***Nonsignificant and significant at probability levels of 0.05, 0.01, and 0.001, respectively.

 \overline{P} ^zNone = control; B, C are the two microbial products.

NS, *, **, ****Nonsignificant and significant at probability levels of 0.05, 0.01, and 0.001, respectively.

 z None = control.

NS, *Nonsignificant, and statistical significance at probability level of 0.05, respectively.

the chemical, physical, or biological soil properties, including soil water characteristics, *Phytophthora* fungus disease populations, or microbial respiration.

Biosolid addition significantly increased the chlorophyll index of citrus leaves in the Indian River experiment, and their N concentration in both experiments (Table 6). In 2005, leaf P was significantly reduced by product B in the Indian River experiment, and leaf Mg was significantly increased in the Ridge experiment by the same product. In 2006, leaf Ca was significantly reduced by product B in the Indian River experiment. Thus, overall, the effects of microbial products on leaf nutrients were not consistent or repeatable. The biosolids treatment tended to significantly reduce leaf concentrations of K and Ca in citrus. Biosolids are inherently deficient in soluble cations such as K^+ and Ca^{2+} due to the dewatering process in the sewage treatment plants and may provide additional adsorption sites for those cations already in the soil.

Tree canopy measurements obtained from the field experiments were all nonsignificant except for an increase in canopy volume from biosolids addition at the Indian River site (Table 7). Fruit yield was not significantly affected by microbial products or biosolids (Table 8), although a nearly significant (*P* < 0.062) response was noted at the Ridge site in the 2005–06 season, but only between the two microbial products. The microbial products and biosolids did not increase fruit yields or any fruit quality parameters above the levels of the control. Due to the small non-repeatable fruit yield response and a lack of response in other yield components such as canopy growth, we concluded that the microbial products could not consistently improve yield in field-grown citrus under Florida conditions.

Conclusions

In initial greenhouse studies, total plant growth tended to increase in response to soil amendments of two products (B, C), but growth responses to increased rates of B and C were not conclusive. Nitrogen leaching losses and leaf N responses were not remarkable. The other two products (A, D) had no effect on seedling growth or N budgets. Greenhouse experiments were repeated twice in two subsequent years at high and low nutrition rates using B and C, but seedling growth and mineral nutrient status were little affected.

zAnalysis of covariance (ANCOVA) was used for yield analysis, so that the original variability of tree size in the grove was accounted for. $YTSS =$ total soluble solids (lb soluble solids per box \times boxes per acre).

 x None = control; B, C are the two microbial products.

 $NS =$ nonsignificant.

In three years of field tests, no consistent or significant responses to the microbial products were detected in the soil or citrus crop. The only consistent positive responses in the field experiments were from the biosolids treatments, confirming that the experiments were sufficiently sensitive to detect real differences in the field. These results from testing microbial products on citrus were in general agreement with other studies on Florida potatoes. Based on these results, the additional cost and labor of incorporating these microbial products into a Florida citrus production program could not be justified.

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