The Use of Organic Biostimulants to Help Low Input Sustainable Agriculture

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ABSTRACT. The use of biostimulant compounds in forestry and agriculture offers significant opportunity for farmers, according to findings from current university research and field trials. Improved root and shoot growth, better stress resistance, better root growth potential, and reduction in nitrogen levels of fertilization are some of the possibilities that these compounds connote to sustainable agriculture.

INTRODUCTION

The March, 1986 report of the Congressional Office of Technology Assessment entitled *Technology, Public Policy, and the Changing Structure of American Agriculture* states: "Although organic farming maintains soil quality better and reduces contamination of air, water, soil, and final food products, much research is needed to determine how to maximize the integration of organic practices" (U.S.O.T.A., 1986). World demand for agricultural products, especially food, is great. New insights and techniques are required in order to achieve sufficient and sustainable yields to meet global food demand and prevent world hunger. The traditional

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chemical fertilizers have been studied for almost 200 hundred years. It is unlikely that dramatically better chemical fertilizers can be constructed. However, if we are to provide food, fiber, shelter, and fuelwood for the world's burgeoning population, methods of increasing fertilizer efficiency must be investigated.

One approach to increasing crop productivity is the development of non-polluting organic biostimulants (OB). These compounds increase plant growth and vigor through increased efficiency of nutrient and water uptake. Definitions for biostimulants vary greatly and there is still some arguments surrounding these compounds. However, they are defined as non-fertilizer products which have a beneficial effect on plant growth. Many of these biostimulant materials are natural products that contain no added chemicals or synthetic plant growth regulators. The initial empirical image of these compounds is changing. Major scientific research in universities such as Clemson, Fairfield, Mississippi State, Virginia Polytechnic Institute, and Yale is demonstrating that under certain conditions biostimulants work well and open significant production possibilities in sustainable agriculture. Also, biostimulants are being used increasingly in horticulture and silviculture.

THE COMPONENTS OF OUR ORGANIC BIOSTIMULANT

Our research at the Yale University School of Forestry and Environmental Studies has developed, with the support of Soilizer Corporation (25 Science Park, New Haven, Connecticut), a new biostimulant (ROOTS™). The product consists of a mix of humic acids, marine algae extracts, a non-hormonal reductant plant metabolite, and B vitamins. This blend greatly increases root and top growth of plants, while decreasing fertilizer requirements up to 50% in a number of species (coffee, several grass species, pines, Douglas-fir, *Alnus, Gliricidia*). The biostimulant also increases resistance to stresses such as low soil water potential and possibly residual herbicides in soil (Berlyn and Beck 1980).

The effect of the individual components of the biostimulant to promote plant growth have been studied by many researchers (Booth 1966, Senn and Kingman 1973, Hernando 1968, Berlyn and

Beck 1980, Metting 1985, Oerli 1987). However, the innovation of mixing them and capitalizing on their synergistic effects is a real contribution in terms of agricultural production.

An overview of some of the individual components of the biostimulant blend is as follows:

Humic Acids—Humic substances comprise 65-70% of the organic matter in soils (Hernando 1968). These compounds are the product of the decomposition of plant tissues, and they are predominantly derived from lignified cell walls. They consist of both humic acids and fulvic acids. The major functional groups of humic acids, HA, include carboxyls, phenolic hydroxyls, alcoholic hydroxyls, ketones, and quinones. The mechanism of HA action in promoting plant growth is not completely known. Several explanations have been given such as: (1) increase in cell membrane permeability (Hewitt 1952, Hernando 1968, Visser 1985); (2) increase in oxygen uptake, respiration and photosynthesis (Aitken, Acock and Senn, 1964; Hernando, 1968; 1975); (3) increase in phosphorus uptake (Jelenic et al. 1966); (4) increased root and cell elongation (Aso and Yamaguchi 1971, Schnitzer and Poapst 1971; Vaugham 1974); (5) increased ion transport (Cacco and Civelli 1973); and (6) acting as cytokinin-like substances (Cacco and Dell'Agnolla 1984). There are other hypotheses about the function of humic acids but these represent the current consensus.

Marine Algae—This is a commonly used organic supplement for increasing plant growth and stress resistance (Young and McLachlan 1966, Booth 1966, Tay et al. 1987, Metting 1985, Senn 1987). A well documented active ingredient of marine algae is cytokinin content (Hofman et al. 1986, Tay et al. 1987). The major seaweed used in our blends is *Ascophyllum nodosum*. According to Senn (1987) the main cytokinins found in such algae are adenine and zeatin. Often the algal material is sold with a certain guaranteed cytokinin content such as 100 ppm, but this can vary with species, season, and extraction procedures. The working concentration in our biostimulant is ca. 0.1 ppm, which is low, but within the lower range of biological effect. Known cytokinin effects include: wound healing, delay of senescence and chlorosis, increased chloroplast development, promotion of cell division, organ formation (especially in tissue culture), and stimulation (or sometimes inhibition) of cell enlargement (Horgan 1984, Salisbury and Ross 1985). Moreover, cytokinins have been shown to increase root elongation and root hair development (Abutylbov and Akundova 1982, Bittner and Buschmann 1983, see also Clarkson 1985). Root hairs represent a way to greatly increase absorptive surface and are thought by some to have enhanced respiratory capacities that promote active ion uptake (Bhaskar and Berlyn 1988). Some of these cytokinin effects are similar to those manifested by the OB. However, these effects are also manifested in the absence of the marine algal component and thus in the absence of cytokinin.

SOME PRELIMINARY GREENHOUSE AND FIELD STUDIES

A preliminary study was aimed to test different concentrations (dilutions) of the biostimulant (ROOTS™) on growth of loblolly pine and Douglas-fir seedlings. Results showed that in both species the best responses were obtained with a 1% solution. Since obtaining these results, almost all subsequent greenhouse experiments and field trials have been done with water dilutions of 50 to 1 and 100 to 1. In tissue culture and hydroponic work a concentration of 1000: 1 is used.

The Effect of ROOTS™ on Chlorophyll Content of Rye-Grass (Lolium perenne)

The combined effect of humic acids, marine algae, and "metab" (a proprietary intermediate metabolite) on the chlorophyll content in rye-grass (*Lolium perenne*) was tested in the following experiment. Three treatments were applied once a week: (1) 50 ml of tap water (control); (2) 50 ml of a 1% solution of a mix (1:1 vol.) humic acid from leonardite and marine algae (*Ascophyllum nodusum*); and (3) 50 ml of a 1% solution of a mix (1:1 vol.) humic acid from leonardite and marine algae plus "metab" (3%) once a week. All the pots were regularly watered three times a week. The 50 ml treatment essentially saturated the soil in the pots. Chlorophyll content was determined using the method of Hiscox and Israelstam (1979) in which chlorophyll is extracted from plant tissue with dimethyl sulfoxide (DMSO) without maceration. Seven weeks after sowing, 6 pots were selected from each treatment and 500 mg of fresh leaf tissue from each pot were taken for analysis. The leaves were rinsed in distilled water and immediately placed in DMSO and heated to 65°C for 45 minutes in a water bath. The extract was cooled at room temperature, and the concentration of chlorophyll in the DMSO was determined by measuring absorption at 645 and 663 nm in a spectrophotometer. The Arnon (1949) equation was used to convert the absorption values to mg per liter of solution. Total chlorophyll (mg/liter) = (20.2 × O.D.645) + (8.02 × O.D.663); where O.D.645 and O.D.663 are the optical densities at 645 and 663 nm. Dry weight percentage of samples from each treatment were determined after drying samples for 96 hours at 70° C. All values were finally converted to milligram of chlorophyll per gram of dry leaf. Data were processed for analysis of variance.

The data show that both the (HA + MA + Metab) and the (HA + MA) treatments had statistically significantly higher chlorophyll content than the control. However, the addition of Metab greatly enhanced chlorophyll content over that of (HA + MA). Statistical differences ($P < 0.01$) exist among the three treatments as presented in Figure 1. The (HA + MA) treatment yielded 74% more chlorophyll than the control. The (HA + MA + Metab) combination yielded 207% more than the control and 76% more than just (HA + MA). Thus, (HA + MA + Metab) was used as the basis for ROOTS™.

After the chlorophyll content experiment was completed, all of the rye grass plants were mowed to a height of 4 cm. Subsequently the plants were kept watered, but all HA + MA and HA + MA + Metab treatments were discontinued. The difference in plant height was measured 12 days later and the results are shown in Figure 2. Clearly there was a residual effect of the treatments with HA + MA + Metab > HA + MA > C. The means were significantly different and in addition visual observation indicated that the chlorophyll content had the same trend as the mean height.

FIGURE 1. Chlorophyll content in rye-grass *(Lolium perenne)* treated with humic acid (HA) + marine algae (MA) 1% solution and HA + MA + "metab" (3%) (ROOTS™) solution once a week.

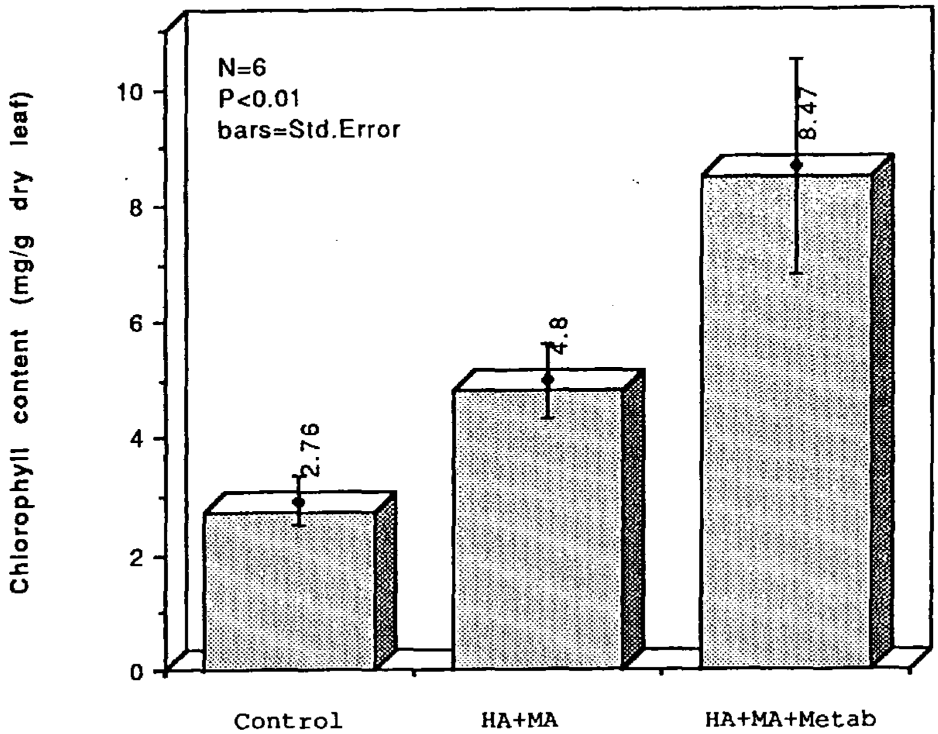
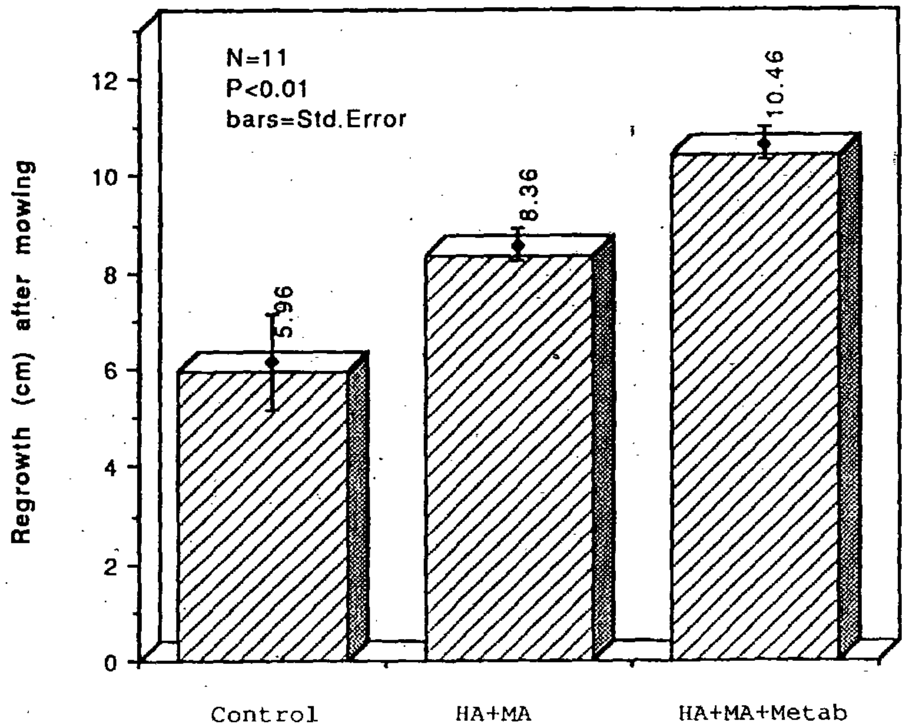


FIGURE 2. Residual effect of the treatments with humic acid (HA) + marine algae (MA) 1% solution and HA + MA + "metab" 3% (ROOTS™) after mowing and cancellation of treatments.



Root Regeneration Capacity of Black Walnut Seedlings Treated with ROOTS™ Under Greenhouse Conditions

This experiment was performed to test the effect of ROOTS™ on root growth potential (RGP) or root regeneration capacity. This is a measure of the ability of a bare-root seedling to produce new roots (Feret, Kreyman and Kreb, 1985), and has been shown to be a valuable index of seedling quality (Ritchie and Dunlap, 1980). Seedlings of Black Walnut (third year growth) were lifted from the Patchaug State Nursery, Voluntown, Connecticut.

Forty plants were root-pruned at 13-14 cm and lateral roots were removed; they were planted in individual half gallon pots. Plants were randomly arranged and half received a solution 2% ROOTS™, 200 ml/plant. After ten weeks of growth, stem length and diameter were measured. Root length and diameter of the old remaining root were also measured. New roots were excised and were oven-dried to evaluate dry weight of new roots produced. The number of new branches and number of leaves were recorded before obtaining shoot dry weight.

Results showed that treated seedlings had 42% higher leaf dry weight and 94% higher root dry weight values than untreated control seedlings. No statistically significant differences were shown in new stem growth (see Table 1a).

The Effect of ROOTS™ on Root Growth of Young Sod

The objective of this field test was to evaluate the effect of the biostimulant on root growth of a young sod. The test was performed at Delea and Sons Sod Farm, East Northport, Long Island, New York. On May 11, 1987, a mix of Jaguar Tall Fescue (*Festuca* sp.) 90% and Challenger Bluegrass (*Poa pratensis*) 10% was sown on a sandy loam soil. Fall and Spring applications of ROOTS™ were applied on a two acre plot of the sod in November 1987 and April 1988, at a rate of 1 gallon/acre per application. On July 10, 1988, three samples (6 × 11 cm) from the treated and untreated plots were taken at random. Fresh and dry weight of shoot and root clip-

TABLE 1a. Leaf and root regrowth of black walnut seedlings, seventy days after root pruning and transplanting.

	ROOTS™	CONTROL	INCREMENT
	---mg/seedling---		
Leaf dry weight	4961.5	3484.5	42% *
Root dry weight	1015.6	521.4	94% *
New Stem dry weight	765.3	614.7	24% ns

N=20; *increment differences were significant at 95% level pings were evaluated for each sample. To evaluate root depth, five measurements were made in each treatment.

Results showed that the root fresh weight of the treated sod was 65% higher ($P < 0.01$) than the untreated control plots. The root dry weight of the treated sod was 59% higher ($P = 0.03$) than the untreated control plots. Root water content of treated plants (61%) was no different from untreated grass (63%). However, shoot water content was higher in treated grass (79%) than that found in untreated grass (72%). The fresh and dry weights of shoot clippings were not significantly different. The mean root depth was 35% higher in the grass treated with ROOTS™. From these results it can be concluded that the grass treated with ROOTS™ demonstrated significantly more root mass development than the control grass (see Figure 3).

The Effect of ROOTS™ on Root Growth of a Bentgrass Putting Green in Field Conditions at the Yale University Golf Course, New Haven, Connecticut

In this field test the objective was to evaluate the effect of ROOTS™ on root growth of Southern German Bentgrass seeded in 1924. The test was performed on green #16 of the Yale Golf Course, New Haven, Connecticut. Half of the grass received 4 ounces/1000 sq.ft. of ROOTS™ as a liquid solution diluted 50:1 in water, on April 19, 1989; the other half of the green was left untreated as a control. Fourteen core samples from each plot were randomly taken with a 3/4" diameter borer up to 4" depth on June 26, 1989. The samples were washed and oven-dried to obtain root dry weight.

Results showed that Southern German Bentgrass treated with ROOTS™ showed 56% more root dry weight than the untreated control samples. This difference was statistically significant at 95% level ($P < 0.05$). All treated samples showed more root depth and development than untreated samples (see Figure 4).

FIGURE 3. Root dry weight of a fescue/bluegrass mix treated for six months with one gallon per acre of ROOTS™ in two applications (November 1987 and April 1988).

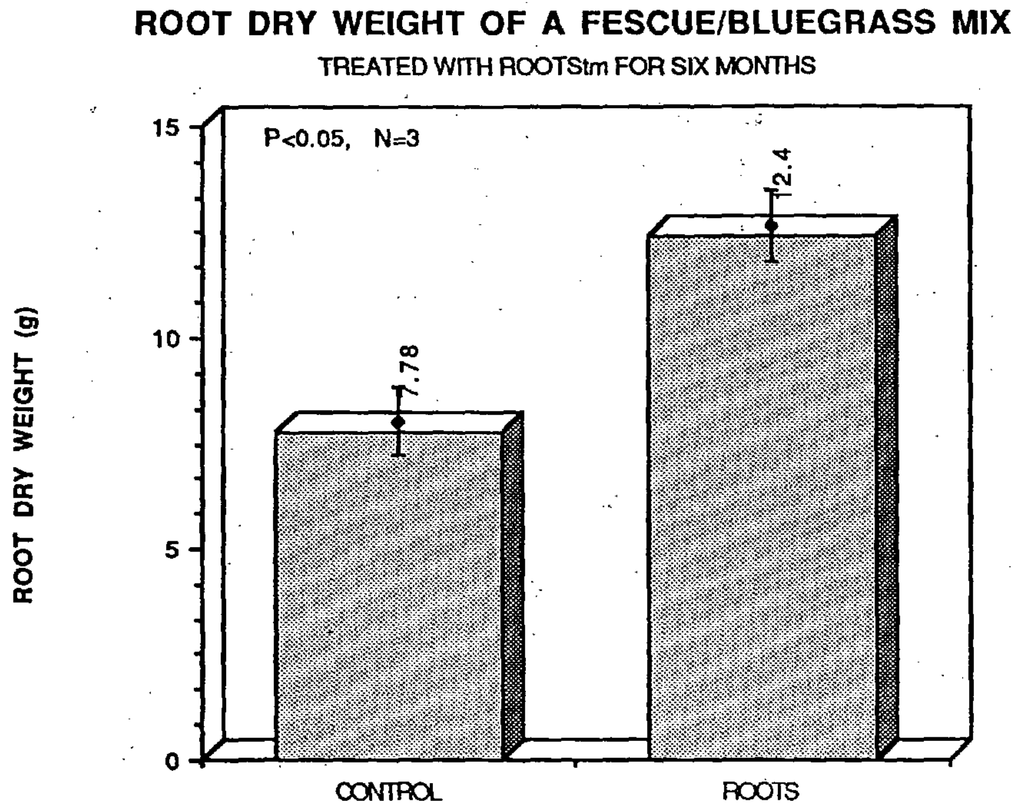
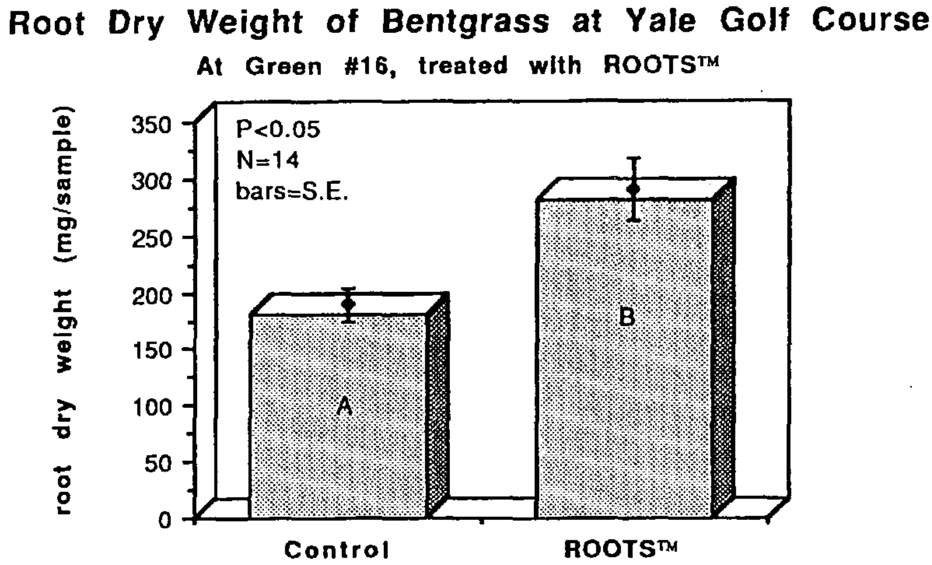


FIGURE 4. Root dry weight in a bentgrass putting green treated once on April 19, 1989 with 50:1 dilution of ROOTS™. Plants were harvested on June 26, 1989.



The Effect of ROOTS™ on Growth of Loblolly Pine Seedlings in Nursery Conditions at St. Joseph Nursery, Capps, Florida

A field test was performed at the St. Joseph Land and Development Corporation Capps Nursery, in Florida. The objective was to evaluate the effect of ROOTS™ on loblolly pine (*Pinus taeda*) seedlings under nursery conditions. A test area of 1000 sq. ft. of nursery beds was treated with a solution of ROOTS™ (1:50 water by volume) applied in five applications during the active growing season. In November 1988, a sample of treated and untreated adjacent plants were harvested and shipped cold to New Haven, Connecticut for analysis. One hundred seedlings from each group were randomly chosen and weighed for stem and root fresh and dry weights. Stem diameter and total height were also measured. Data were analysed with Statview II for one factor analysis of variance for repeated measures.

Results showed that treated seedlings had a significant increase of both fresh and dry weights of shoot and root biomass. In both cases the increases were greater in fresh weight, indicating ROOTS™ also increased water uptake (see Table 1b). (Separate tests showed that treated plants have increased xylem volume and conductivitys.)

The Effect of ROOTS™ on Growth of Loblolly Pine Seedlings in Nursery Conditions at Stilman Nursery, Jasper, Texas

Another field test was performed at the Stilman Nursery (Southwestern Timber Company, a Division of Eastex Corporation) in Jasper, Texas. The objective was to evaluate the effect of ROOTS™ on nursery seedlings. The test was performed on loblolly pine (*Pinus taeda*) in nursery field conditions. Two treatments were considered: (1) A test plot of nursery beds without ROOTS™ treatment; and (2) A test plot of nursery beds was treated with a solution of ROOTS™ (1: 50 water by volume). Two applications of ROOTS™

TABLE 1b. Shoot and root growth of Loblolly Pine treated with ROOTS™ in St. Joseph Nursery, Capps, FL.

	ROOTS™	CONTROL	INCREMENT
Stem Diameter (mm)	4.76	3.65	1.11**
Shoot Fresh Weight (g)	15.02	7.99	7.03**
Shoot Dry Weight (g)	3.94	2.23	1.71**
Root Fresh Weight (g)	2.56	1.07	1.49**
Root Dry Weight (g)	0.67	0.33	0.34**

N=100; ** increment differences were significant ($P<0.01$)

were made during the active growing season. A sample of treated and untreated adjacent plants were harvested and sent cold to New Haven, Connecticut for analysis. Fifty seedlings from each group (treated and untreated) were randomly chosen and weighed for shoot and root fresh and dry weights. Stem diameter was also measured. Data were analysed with Statview II for one factor analysis of variance for repeated measures.

Results showed that seedlings treated with ROOTS™ had statistical differences ($P < 0.05$) in diameter and shoot dry weight compared with those without ROOTS™ (see Table 2). However, all treated plants showed higher means than non-treated seedlings (P values for the F test are Table 1).

The Effect of ROOTS™ on Growth of Red Maple Seedlings in Nursery Conditions at Superior Trees Nursery, Lee, Florida

A field test was performed at the Superior Trees, Inc. Nursery in Lee, Florida; the objective was to evaluate the effect of ROOTS™ on nursery seedlings. The test was performed on red maple (*Acer rubrum*) seedlings in operational nursery field conditions. A test area of 1000 sq. ft. of nursery beds was treated with a solution of ROOTS™ (1: 50 water by volume) applied in five applications during the active growing season. In November 1988, a sample of treated and untreated adjacent plants were harvested and sent cold to New Haven, Connecticut, for analysis. Thirty-seven seedlings from each group were randomly chosen and weighed for stem and root fresh and dry weights. Stem diameter and total height were also measured. Data were analysed with Statview II for one factor analysis of variance for repeated measures.

Results showed that treated seedlings had higher values of stem diameter, total height and fresh weights of shoot and root biomass. All the increments were significant, but the root fresh and dry weight increments showed the highest percentage increase (see Table 3).

TABLE 2. Shoot and root growth of Loblolly Pine treated with ROOTS™ in the Stilman Nursery in Jasper, Texas.

	ROOTS™	CONTROL	P value
	Weight (g)		
Shoot fresh weight	10.31	8.93	0.0761
Root fresh weight	2.51	2.09	0.1742
Shoot dry weight	3.23	2.77	0.0450*
Root dry weight	0.78	0.70	0.3739
Diameter (mm)	4.77	4.38	0.0111*

N=50

TABLE 3. Shoot and root growth of red maple treated with ROOTS™ in Superior Trees, Inc. Nursery in Lee, FL.

	ROOTS™	CONTROL	INCREMENT	INC%
Stem Diameter (mm)	6.20	4.94	1.26	25.5
Stem Height (cm)	29.95	23.40	6.55	28.0
Stem Fresh Weight (g)	2.52	1.40	1.12	80.0
Root Fresh Weight (g)	5.88	3.19	2.69	84.3
Stem Dry Weight (g)	1.41	0.81	0.60	74.1
Root Dry Weight (g)	2.19	1.19	1.00	84.0

N=37; all differences were statistically significant ($P<0.01$)

The Effect of ROOTS™ on Growth of Loblolly Pine Seedlings in Nursery Conditions at Mead Corporation, Coated Board Division Nursery, Buena Vista, Georgia

A field test was performed at the Mead Corporation, Coated Board Division Nursery in Buena Vista, Georgia. The objective was to evaluate the effect of ROOTS™ on nursery seedlings. The test was performed on loblolly pine (*Pinus taeda*) in nursery field conditions. Two treatments were considered: (1) a plot of nursery beds without ROOTS™, the control treatment; and (2) a plot of nursery beds sown treated with a solution of ROOTS™ (1: 50 water by volume). Two applications of ROOTS™ were made during the active growing season. In February 1989, a sample of treated and untreated adjacent plants were harvested and sent cold to New Haven, Connecticut for analysis. Fifty seedlings from each group (treated and untreated) were randomly chosen and weighed for shoot and root fresh and dry weights, as well as stem diameter. Data were analysed with Statview II for one factor analysis of variance for repeated measures.

Seedlings treated with ROOTS™ showed comparable shoot and root growth as those sown two weeks before without ROOTS™. In addition, the greater root dry weight of ROOTS™ treated seedlings were significantly higher than the root dry weight of control seedlings sown at the same time (Table 4).

The Effect of ROOTS™ on Growth of Sand Pine Seedlings in Nursery Conditions at Buckeye Nursery, Perry, Florida

A field test was performed at the Buckeye Cellulose Corporation Nursery in Perry, Florida. The objective was to evaluate the effect of ROOTS™ on sand pine (*Pinus clausa*) seedlings under nursery field conditions. A test area of 1000 sq. ft. of nursery beds was treated with a solution of ROOTS™ (1:50 water by volume). Two applications of ROOTS™ were made during the active growing season for each species. In November 1988, a sample of treated and untreated adjacent plants were harvested and sent to Yale in cold

TABLE 4. Shoot and root growth of Loblolly Pine treated with ROOTS™ in the Mead Corporation, Coated Board Division Nursery in Buena Vista, Georgia.

	CONTROL	ROOTS™
	------Dry Weight (g)------	
Shoot	2.18*	2.29*
Root	0.98*	1.38*

N=50; * $P<0.05$

conditions for analysis. Fifty seedlings from each group (treated and untreated) were randomly chosen and weighed for shoot and root fresh and dry weights. Stem diameter was also measured. Data were analysed with Statview II for one factor analysis of variance for repeated measures.

Sand pine treated seedlings showed higher values of both shoot and root fresh weights. Loblolly pine showed no differences in shoot increments but root increments were significant (see Table 5).

AN OVERVIEW ON LOBLOLLY PINE NURSERY FIELD RESEARCH

Based on the results of the forestry nursery tests, it can be concluded that the biostimulant, ROOTS™, showed a positive effect on nursery seedling growth.

In the particular case of loblolly pine, 22% more dry weight of shoots (and up to 77% in one case) and 32% more root dry weight (reaching more than 100% in some cases). These results, based on means, were obtained at six of the large forestry nurseries of the Southeastern United States under operational conditions (Table 6).

CONCLUSIONS

This organic biostimulant, ROOTS™, that we have developed and tested seems to offer a significant opportunity to increase plant growth, according to findings from current university research and field trials. Improved root and shoot growth, better root growth potential, and better stress resistance seem to be consistent with results obtained from using this OB. But the most important possibility for the future of this organic biostimulant, may be its ability to cut down chemical fertilizer without affecting growth. Preliminary research done by Russo (1989, unpublished) showed that in the presence of the biostimulant, coffee seedlings treated with the half amount of fertilizer yielded the same shoot biomass and higher root biomass than those fully fertilized. These investigations are continuing.

TABLE 5. Shoot and root growth of Sand Pine treated with ROOTS™ in Buckeye Cellulose Corporation Nursery in Perry, FL.

	ROOTS™	CONTROL	INCREMENT	INC.
Shoot Fresh Weight (g)	5.50	3.96	1.54*	39%
Root Fresh Weight (g)	0.58	0.33	0.25**	76%
Diameter (mm)	2.74	2.21	0.53**	24%
Shoot Dry Weight (g)	1.38	0.96	0.42*	44%
Root Dry Weight (g)	0.17	0.10	0.07*	70%

N=50; * P<0.05; ** P<0.01; ns not significant

TABLE 6. Root growth response to ROOTS™ of Loblolly Pine in six Southeastern nurseries.

Nursery		Root Dry Weight (%)
Saint Joseph FL	Control	100
	Treated	203
Stilman TX	Control	100
	Treated	111
Mead GA	Control	100
	Treated	141
International AL	Control	100
	Treated	109
Federal SC	Control	100
	Treated	115
Buckeye FL	Control	100
	Treated	107
Means	Control	100
	Treated	130

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