Proc. Fla. State Hort. Soc. 130:240–244. 2017.

Biostimulants Have Minimal Effects on Growth of Some Woody Nursery Plants

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Additional index words. live oak, *Quercus virginiana* Cathedral Oak™ , elm, *Ulmus parvifolia* 'Allee', Leyland Cypress, *Cupressocyparis leylandii*, On-Gard, Bioflourish, Helena Nutri-Health, transplant shock, biostimulant

Biostimulants are often marketed to improve rooting and growth of nursery plants. An experiment was initiated to evaluate the effectiveness of four commercial biostimulants on growth of three common nursery plants: Leyland cypress (*Cupressus* x*leylandii*), Cathedral Oak™ live oak (*Quercus virginiana*), and 'Allee' elm, (*Ulmus parvifolia*). Liners were potted in #3 Air-Pots® containing a peat-pine bark substrate in June 2015 and grown under full sun in central Florida. Ten days after potting, plants were treated with one of four biostimulants either as foliar spray and/or soil drench every two weeks as directed on the label: Helena plant health program, Pathways biostimulant, Bio Flourish, and BioWorks On-Gard. Untreated plants were maintained as controls. The experiment was arranged as a completely randomized design with 10 replications per treatment for each species. Plants were managed by a local commercial tree farm with the same cultural practices applied to their nursery plants. Plant height, width, and caliper were taken at initiation and eight months later at the conclusion. At the end of the experiment, five randomly selected plants from each treatment were washed clean, divided into roots and shoots, oven dried, root and shoot dry weights recorded, and root: shoot ratios calculated. Results showed that although some measured parameters differed among treatments, only BioWorks On-Gard significantly increased plant height of 'Allee' elm and Pathway biostimulant significantly increased the growth index of Leyland cypress compared to respective control plants. These results suggest that positive effects of biostimulants on plant growth may be plant species specific.

Transplanting causes a slowing of plant growth as roots recover from the stress of being removed from the pot and placed in a new environment. Each time growers move plants to different pots or different locations, plant growth is impacted by transplant shock. Biostimulants promise to reduce this stress, although matching the biostimulant with the plant species is required for success (Barnes and Percival, 2006; Fraser and Percival, 2003), few experiments have been conducted on woody ornamental crops in pots (Calvo et al. 2014).

Biostimulants are biologically or naturally-derived additives that promote plant growth. The North American Biostimulant Coalition defines them as "any substance or compound other than primary, secondary, and micro plant nutrients that can be demonstrated by scientific research to be beneficial to one or more species of plants, when applied exogenously" (Biostimulant, 2016).

Various commercial biostimulant products are available and marketed with claims that may or may not have been substantiated in the public domain. In a review of agricultural uses of plant biostimulants, Calvo et al., 2014, report that some products work with some plants under some conditions, but not all plants at all times, and mechanisms were poorly understood. The market for biostimulants is expected to increase dramatically, especially with the interest in organic production and the potential for biostimulants to sustainably increase yield and productivity (Chandrasekhar, 2016). Biostimulants can include, but are not limited to microbial inoculants, humic acid, fulvic acid, protein hydrolysates, amino acids, micronutrients, and seaweed extracts.

To assess potential roles of biostimulants in alleviation of transplant shock of ornamental trees, we evaluated the effects of four commercially available biostimulants on the growth of three woody plant species for eight months after transplanting liners into #3 containers. The tested commercial products provided a range of biostimulants from seaweed, humates, protein hydrolysates, microbials, and micronutrients, some having a mixture of all types, and others having only one. Results from this work will provide plant producers with a greater understanding of biostimulant use efficacy.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the University of Florida and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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Materials and Methods

Liners of Cathedral Oak™ live oak (*Quercus virginiana*), Leyland cypress (*Cupressus ×leylandii*), and Allee elm (*Ulmus parvifolia*), were obtained from a local nursery and transplanted 9 June 2015, into 10-inch diameter, 11.5-inch tall Air-Pots® (Caledonian Tree Co. Ltd, Midlothian, Scotland) using a woody ornamental mix of 40% peat, 60% 3/8-inch pine bark, with 75 lb Micro Max Micro nutrient blend (Everis, Geldermalsen, The Netherlands), 100 lb Dolomite, and 1100 lb 21–4–8 (12–14 month) per 60 yd^3 increment (Everis, Geldermalsen, The Netherlands). Plants were arranged as a completely random design with 10 replications (10 trees per treatment) and each plant genus was considered a separate experiment. On 19 June 2015, the first treatments were applied and every 2 weeks thereafter until the conclusion of the experiment on 12 Jan. 2016. Treatments included Pathway, Bio-Works On-Gard, Bioflourish, or Helena Nutri-Health (Tables 1–4). Untreated plants were maintained as controls. Plants were grown at the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Mid Florida Research and Education Center in Apopka, FL, and maintained by a local nursery using their cultural practices.

Table 1. Composition of Pathway biostimulant as reported on the label.

Ingredient	Amount
Drench:	
Manage Fungi	1 oz/50 gal
Microbial Ingredients	3%
Trichoderma harzianum	PB 071 5 x 10^7 cfu/g
Trichoderma viride	PB 072 5 x 10^7 cfu/g
Trichoderma longibrachiatum	PB 006 5 x 10^7 cfu/g
Endomycorrhizal fungi	
Glomus intraradices	550 propagules/g
Glomus mosseae	550 propagules/g
Glomus aggregatum	550 propagules/g
Glomus etunicatum	550 propagules/g
Glucose based culture media	96%
ENRG:	1 pt/50 gal
Fulvic Acid	5.0%
Kelp (*Ecklonia maxima*)	4.0%
Kelp (*Ascophyllum nodosum*)	4.0%
Amino acids: glutamic acid, glycine, alanine, proline, arginine, leucine, valine, lysine, serine, phenylalanine, aspartic acid, threonine, isoleucine, tyrosine, histidine, methionine, taurine, cystine, tryptophan (obtained from fish hydrolysate by the hydrolyzation of proteins to their constituents amino acids)	20%
Maltodextrin	10%
Molasses	10%
Spray:	
Merge Microbial Inoculant	1 pt/50 gal
Bacillus subtilis	PB 038 5 x 10^8 cfu/mL
Bacillus subtilis	PB 346 5 x 10^8 cfu/mL
Bacillus methylotrophicus	PB 105 5 x 10^8 cfu/mL
Bacillus methylotrophicus	PB 302 5 x 10^8 cfu/mL
Bacillus amyloliquefaciens	PB 178 5 x 10^8 cfu/mL
Bacillus amyloliquefaciens	PB 390 5 x 10^8 cfu/mL
Bacillus megaterium	PB 208 5 x 10^8 cfu/mL
Bacillus licheniformis	PB 035 5 x 10^6 cfu/mL
Kelp (*Ascophyluum nodosum*)	2%
Glucose based culture medium	88%

Table 2. Composition of Bio Flourish as reported on the label.

Ingredient	Amount
Drench:	
Bioflourish:	
Proprietary Lactic Acid Bacteria	3.02 x 10^5 cfu/mL
Organic Sugar Cane Molasses	Not listed
Sea Salt	Not listed
Yeast	1.32 x 10^5 cfu/mL
Formula One	
Phosphite Fertilizer 0–29–26	1% 6.4 oz/5 gal
Spray:	
Acadian Stimplex:	1.5 pt/100 gal
Cytokinin (as Kinetin) extracted from marine plants	0.01%

Table 3. Composition of Helena Nutri-Health biostimulant as reported on the label.

Ingredient	Amount
Drench:	
Asset RTU	32 oz/100 gal
Viva	32 oz/100 gal
Utilize	10 oz/100 gal
Hydrahume	32 oz/100 gal
Axilo5	16 oz/100 gal
Soakerplus	10 oz/100 gal
Spray:	
Coron	64 oz/100 gal
Ele-max	64 oz/100 gal
Utilize	10 oz/100 gal
Tracite Fe	32 oz/100 gal
Cohere non-ionic spreader-sticker	10 oz/100 gal
Brexil multi	16 oz/100 gal
Renova	32 oz/100 gal

Label rates were used on all treatments. The Pathway (Pathway Biologic LLC, Plant City, FL) treatment consisted of a 16 oz drench and a spray of ≈ 0.17–0.255 oz/plant (Table 1) every two weeks. BioWorks ON-Gard (Bioworks Inc., Victor, NY) is a fertilizer based 100% on enzymatically extracted plant-derived amino acids (30%) with 5.0% total nitrogen and 2.0% soluble potash. Our treatment consisted of a 16 oz drench of ON-Gard at a rate of 2 qt/100 gal every two weeks. The Bio Flourish treatment (Triangle C. C., Macon, GA) consisted of a 16 oz drench of 1% Formula One and 2% Bioflourish, and a spray of ≈ 0.17–0.255 oz $plant^{-1}$ of Acadian Stimplex at 1.5 pt/100 gal (Table 2). The Helena Nutri-Health (Helena Chemical Company, Collierville, TN) treatment consisted of a 16 oz drench every other week, and a ≈ 0.17–0.255 oz/plant spray applied twice during the experiment on 19 June 2015 and 20 Oct. 2015 (Table 3).

Plants were staked, tied, and a pre-emergence herbicide (Freehand®, BASF, Research Triangle Park, NC) applied on 30 June 2015. Plants were managed with the same cultural practices as a local commercial tree farm typically applies to their nursery plants except for the application of the stimulants. On 18 Sept. 2015, the elms and oaks were pruned as needed for form, attached to an overhead trellis wire, and all pots received a second pre-emergent herbicide application (Freehand®). Pruning was limited to selecting a single leader without removing lower non-competing limbs and performed by the nursery. The containers were placed on polypropylene ground cover under full sun conditions in central Florida and watered daily using overhead irrigation.

Table 4. Effect of biostimulants on Cathedral Oak™ live oak liner growth.

Treatment	Change in height (cm)[z]	Caliper change at 15 cm (cm)[z]	Change in growth index	Root dry weight (g)[z]	Shoot dry weight (g)[z]	Root:Shoot ratio
Control	23.6 ab[x]	2.9 a	900.6 ab	8.57 a	15.02 a	0.61 a
Helena	30.0 a	3.0 a	1258.1 ab	10.12 a	19.42 a	0.55 a
On-Gard	24.6 ab	4.2 a	782.9 ab	15.06 a	25.00 a	0.58 a
Pathway	27.2 ab	3.5 a	1155.6 ab	14.04 a	23.62 a	0.61 a
Bio Flourish	16.3 b	3.1 a	609.5 b	10.54 a	15.78 a	0.67 a

[z]1 cm = 0.3937 inches.
[y]1 g = 0.0353 oz.
[x]Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ with Duncan's multiple range test (n=10).

Initial plant height, caliper at 6 in. height, and two canopy widths were recorded on 19 June 2015 and again on 12 Jan. 2016 at the conclusion. A growth index (GI) was calculated as GI = [(canopy widest width + width perpendicular) ÷ 2] × plant height (Stamps et al., 2008) for the oak and the cypress only, as the elms were considered too tall and flexible for width measurements at the conclusion of the experiment. On 12 Jan. 2016 five plants from each treatment were randomly selected, their roots washed and separated from the shoots, and the plant parts dried in an oven at 80 °C for two weeks. Dry weights were measured and root:shoot ratio calculated.

Data for difference between final and initial measurements of plant height, caliper, growth index, root and shoot dry weights, and root:shoot ratio per species were subjected to analysis of variances using SAS (SAS ver. 9.4; SAS Institute, Cary, N.C.). Dunnett's test at $P \leq 0.05$ was used to compare the means of each parameter resulting from each biostimulant treatment to the means of the respective control (Lentner and Bishop, 1986). Data were also subjected to Duncan's Multiple Range Test ($P \leq 0.05$) to determine differences between individual treatment means.

Results and Discussion

Initial measurements before treatment indicated significant differences between plants, therefore the change in height, caliper, and growth index for each plant were calculated and used to compare treatments. The variation seen in the liners before treatment indicate it would be a good practice for nurseries to better grade liners before transplanting. This would provide more uniform plants at finish. The liners were provided and planted for the experiment just as the local commercial nursery practices. Analyzing changes in growth indices for each plant should overcome the problems of initial variation for the purposes of statistical comparison.

The results indicate few significant differences between treatments and the control for the tested species (Tables 4, 5, and 6). A significant increase in growth index from the control was found in cypress with the Pathway treatment (Table 5). BioWorks On-Gard significantly increased plant height of elm compared to the control plants (Table 6). Helena Nutri-Health significantly reduced caliper, root dry weight, and shoot dry weight of elm compared

Table 5. Effect of biostimulants on 'Leyland' cypress liner growth.

Treatment	Change in height (cm)[z]	Caliper change at 15 cm (cm)[z]	Change in growth index	Root dry weight (g)[z]	Shoot dry weight (g)[z]	Root:Shoot ratio
Control	54.7 a[y]	5.7 a	2376.1 b	24.20 a	82.38 a	0.29 a
Helena	55.2 a	6.3 a	2522.1 b	19.40 a	88.82 a	0.22 a
On-Gard	51.0 a	5.8 a	2978.0 ab	12.48 a	92.04 a	0.13 a
Pathway	57.9 a	6.1 a	3023.7 a*	17.06 a	85.84 a	0.20 a
Bio Flourish	53.6 a	5.4 a	2523.0 b	14.5 b*	70.3 a	0.25 a

[z]1 cm = 0.3937 inches, 1 g = 0.0353 oz
[y]Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ with Duncan's multiple range test (Dunnett's test showed the same results as Duncan's test) (n = 10).
*Significant differences by Dunnett's test at $P \leq 0.05$.

Table 6. Effect of biostimulants on 'Allee' elm liner growth.

Treatment	Change in height (cm)[z]	Caliper change at 15 cm (cm)[z]	Root dry weight (g)[z]	Shoot dry weight (g)[z]	Root:Shoot ratio
Control	165.3 b[y]	9.6 a	64.3 a	129.6 a	0.5157 a
Helena	161.5 b	7.7 b*	38.9 b*	74.7 b*	0.5227 a
On-Gard	195.8 a*	8.7 ab	68.6 a	157.9 a	0.4500 a
Pathway	177.9 ab	8.8 ab	66.7 a	122.0 a	0.5431 a
Bioflourish	165.5 b	8. 8 ab	42.3 b*	75.3 b*	0.5725 a

[z]1 cm = 0.3937 inches, 1 g = 0.0353 oz
[y]Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ with Duncan's multiple range test (Dunnett's test showed the same results as Duncan's test) (n=10).
*Significant differences by Dunnett's test at $P \leq 0.05$.

to control, and Bioflourish significantly reduced root and shoot dry weight in elm compared to control (Table 6). Results may also have been affected by the pruning treatments that the nursery imposed on the plants during the growing season. Weight of plant material removed was minimal, but it was not captured or reflected in the dry weights measured at the end of the experiment.

In another study, biostimulants increased only root dry weight in *Camellia japonica* cuttings (Ferrante et al., 2013), and photographs of dried root systems of randomly selected plants from each treatment seemed to indicate that there were visible differences in root systems (Fig. 1), but the statistical analysis of dry weights did not corroborate this observation.

Bio Flourish includes seaweed-extracted cytokinin and microbial inoculants expected to increase root and shoot dry weights. Macroalgae extracts have long been used to improve plant growth. Although the mechanisms for growth promotion have not been fully understood, phytohormones, proteins, amino acids, lipids, minerals, and other substances in the extracts may be responsible for improved plant growth and tolerance of abiotic and biotic stresses (Shekhar Sharma et al., 2014). In this experiment the product had no or negative effects on plant growth.

Microbial inoculants include various free-living fungi, bacteria, and arbuscular mycorrhizal fungi that are still being discovered and isolated for plant growth promotion. They are thought to increase the supply of nutrients to the plant through increased root surface area and nutrient uptake capacity (Vessey, 2003). The commercial microbial formulation, species of plant, type of soil and environmental conditions can affect the success of microbial inoculants in stimulating plant growth through root function, phytohormones, and resistance to abiotic stress (Calvo et al., 2014). Pathway was the most comprehensive mixture of microbials, seaweed extract, and amino acids, and was expected by accounts from growers to be the most effective growth stimulant. These trials did not provide evidence of this.

Humic substances, the result of microbial decomposition and chemical degradation of dead biota, function in controlling nutrient availability, carbon and oxygen exchange in the soil, modulation of rhizosphere beneficial microorganisms, resulting in improved plant growth, yield, and nutrient uptake (Colla et al., 2014). Helena Nutri-Health, containing humates and micronutrients, had no or adverse effects on the plants.

Protein hydrolysates, free amino acids and polypeptides from chemical and/or enzymatic hydrolysis of animal or plant agro-industrial by-products, may enhance plant uptake of nutrients and improve plant growth. Some reports have shown that these substances act similarly to plant hormones to enhance nitrogen uptake and crop performance including increased total dry biomass, leaf nitrogen content and chlorophyll content (Colla et al.,

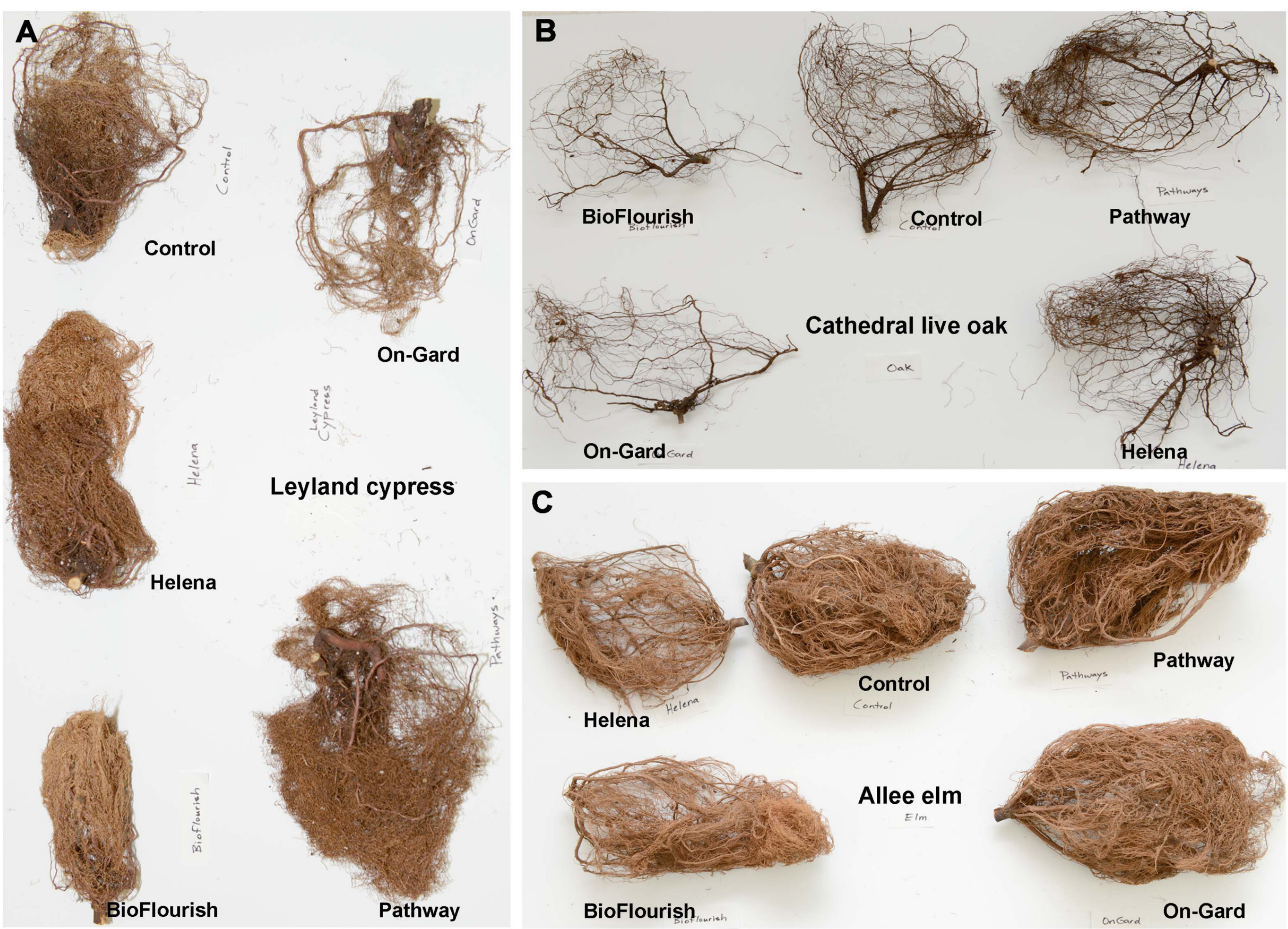

Fig. 1. Root systems of randomly selected Leyland cypress (**A**), Cathedral live oak (**B**), and 'Allee' elm plants (**C**) grown in a substrate composed of 40% peat and 60% pine bark by volume and treated with Helena, On-Gard, Bio Flourish, and Pathway biostimulants along with non-treated control for eight months in Central Florida.

2014). Peptide-based biostimulants increased scion length and leaf chlorophyll concentration, but not stem diameter of mangos in the nursery (Morales-Payan, 2015). BioWorks ON-Gard is this type of product, but in this experiment only improved elm height.

The lack of any growth differences on these three species is puzzling, considering the extensive research showing successful effects of biostimulants on other plants, and anecdotal accounts from growers. Although there are reports of wood ornamental plants not responding to biostimulants at transplanting into the landscape (Abbey and Rathier, 2005; Fraser and Percival, 2003, Sammons and Struve, 2004). In part, microbial biostimulants act by providing better nutrition, protection from diseases, or increased water absorbing capacity (Calvo et al. 2014), especially under suboptimal nutrient conditions (Saa et al., 2015). Lack of disease or pest pressure in this experiment may have influenced the results. Alternatively, soilless media may not provide the type of environment conducive to biostimulants, since research has indicated the original state of the soil can affect results (EBIC, 2013) and most research conducted has not been in pots (Calvo et al. 2014). Results could also be due to the slow growth of these species not allowing differences to show in eight months, although this experiment was much longer than most reported (Calvo et al. 2014). Another alternative is that the living organisms in the treatments applied may not have survived the high-temperature storage conditions of the greenhouse where all treatment containers were stored during the experiment, or the environmental conditions of pots in central Florida weather. More definitive results might be achieved with research performed with larger, graded sample sizes, under more stressful conditions of drought or disease pressure, and with products stored in optimal conditions.

Literature Cited

Abbey, T. and T. Rathier. 2005. Effects of mycorrhizal fungi, biostimulants and water absorbing polymers on the growth and survival of four landscape plant species. J. Environ. Hort. 23(2):108–111.

Barnes, S. and G.C. Percival. 2006. Influence of biostimulants and water-retaining polymer root dips on survival and growth of newly transplanted bare-rooted silver birch and rowan. J. Environ. Hort. 24(3):173–179.

Biostimulant Coalition. 2016. 7 July 2016. <http://www.biostimulant-coalition.org/about/>

Calvo, P., L. Nelson, and J. W. Kloepper. 2014. Agricultural uses of plant biostimulants. Plant Soil 383:3–41.

Chandrasekhar K. 2016. North America biostimulants market by modes of application (foliar, seed, soil) by active ingredient (extracts, acids, others) by end-user (row crops, fruits and vegetables, turfs and ornamentals) by geography-analysis and forecast to 2019. MicroMarket Monitor. 7 July 2016. <http://www.micromarketmonitor.com/market/north-america-bio-stimulants-6850160039.html>

Colla, G., Y. Rouphael, R. Canaguier, E. Svecova, and M. Cardarelli. 2014. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Front. Plant Sci. 5: 448. doi: 10.3389/fpls.2014.00448.

European Biostimulants Industry Council. 2013. Economic overview of the biostimulants sector in Europe. European Biostimulants Industry Council. <http://www.biostimulants.eu/>

Ferrante, A., A. Trivellini, P. Vernieri, and A. Piaggesi. 2013. Application of Actiwave for improving the rooting of camellia cuttings. Acta Hort. 1009: 213–218.

Fraser, G.A. and G.C. Percival. 2003. The influence of biostimulants on growth and vitality of three urban tree species following transplanting. Arboricultural J. 27(1):43–57.

Lentner, M. and T. Bishop. 1986. Experimental Design and Analysis. Valley Book Co., Blacksburg, VA.

Morales-Payan, J.P. 2015. Growth of 'Parvin' mango in nursery as affected by a peptide-based biostimulant applied to the soil. Acta Hort. 1075:161–164.

Saa, S., A. Olivos-Del Rio, S. Castro, and P.H. Brown. 2015. Foliar application of microbial and plant based biostimulants increases growh and potassium uptake in almond [*Prunus dulcis* (Mill.) D. A. Webb]. Front.Plant Sci. 6:87. doi: 10.3389/fpls.2015.00087.

Sammons, J.D., and D.K. Struve. 2004. Effect of Bioplex™ on transplant success of non-dormant red oak (*Quercus rubra* L.). J. Environ. Hort. 22 (4):197–201.

Shekhar Sharma, H.S., C. Fleming, C. Selby, J.R. Rao, and T. Martin. 2014. Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. J. Appl Phycol. 26:465–490.

Stamps, R.H., J.Chen, S. Natarajan, and J. Popenoe. 2008. Evaluations of new controlled-release fertilizers for use in production of interiorscape and landscape plants. Proc. of the Fla. State Hort. Soc. 121:346–349.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586.