Biostimulants and Soil Amendments Affect Two-year Posttransplant Growth of Red Maple and Washington Hawthorn

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Abstract. Humate-based products have been aggressively marketed as biostimulants that increase plant growth. Little data are available on their effect on tree establishment or their interaction with fertilization and irrigation regimes. This experiment tested several types of biostimulants on posttransplant growth of *Acer rubrum* L. (red maple) and *Crataegus phaenopyrum* (Blume) Hara (Washington hawthorn) trees, both with and without irrigation and fertilization. Soil treatments were applied at planting as: 1) control (native backfill only); 2) compost (native backfill + yard-waste compost); 3) peat (native backfill + Canadian sphagnum peat); 4) granular humate, 100 g/tree; 5) granular humate, 200 g/tree; and 6) liquid humate +, a proprietary liquid mixture of humate, kelp extract, thiamine, and intermediate “metabolites.” Irrigation regime $\times$ soil treatment interaction was significant for red maple, but soil treatments did not increase height, stem diameter, top dry mass, or root length. For Washington hawthorn, soil treatments did not increase height, stem diameter, or root length, but top dry mass in all treatments as a group and in humate-treated trees in particular was greater than that of controls. Roots of peat-treated trees of both species were longer than those in other treatments. Granular humate applied at 200 g/tree increased total root length more than did 100 g/tree in Washington hawthorn but not in red maple. Fertilizing at planting with N at 14.5 g·m⁻² had no effect on any parameter measured for either species.

Amendment of planting backfill with organic material does not improve landscape plant establishment in many circumstances (Corley, 1984; Hodge, 1995; Hummel and Johnson, 1985; Smalley and Wood, 1995), although it may improve establishment in specific situations, such as planting in compacted soil (Day et al., 1995) or in mine tailings (Schoenholtz et al., 1992). The decomposition of soil organic matter yields decay-resistant material known as humus (Paul and Clark, 1996). Humic components (humins, humic acid, fulvic acid) increase root growth in sand or solution culture (Lee and Bartlett, 1976; Mylonas and McCants, 1980; O'Donnell, 1973), possibly because they increase nutrient absorption (Rauthan and Schnitzer, 1981; Reynolds et al., 1995; Tan and Nopamobodi, 1979). Humate-based materials are marketed

as biostimulants, described as “non-nutritional products that may reduce fertilizer use and increase yield and resistance to water and temperature stress” (Russo and Berlyn, 1990). Commercial biostimulants may also contain other organic substances, such as vitamins (e.g., thiamine) or seaweed extracts. Improved growth from the application of seaweed extracts may be a result of the presence of auxins and cytokinins (Crouch et al., 1992). Biostimulants have increased growth of field-grown plants in some studies (Poincelot, 1993; Reynolds et al., 1995; Russo and Berlyn, 1990; Sanders et al., 1990), but not others (Albregts et al., 1988; Elliott and Prevatte, 1996; Laiche, 1991).

Rapid establishment of newly planted trees reduces posttransplant mortality and shortens production cycles. Although biostimulant application at planting may increase growth, little information is available concerning its effect on posttransplant growth of nursery trees or its interaction with irrigation and fertilizer practices. Therefore, the objective of this study was to test the effect of various biostimulants and soil amendments, both with and without fertilizer and irrigation, on the establishment of red maple, an easy-to-establish species (Staley and Dickson, 1997), and Washington hawthorn, a difficult-to-establish species (Murakami et al., 1990).

Plant material. One-year-old, bare-root red maple and Washington hawthorn trees (144 of each species) were obtained from Lawyer's Nursery, Plains, Mont., in late Apr. 1995 and held in cold (5 °C) dark storage with roots covered in moist sawdust. All trees were planted in tractor-augured holes ($\approx$ 30-cm depth and diameter) on 10 May 1995 at the Virginia Tech Urban Horticulture Center, Blacksburg, Va. [U.S. Dept. of Agriculture (USDA) hardiness zone 6a] in a Groseclose silt loam soil (clayey, mixed, mesic Typic Hapludults), pH 6.4, NO₃-N at 45 mg·kg⁻¹. Initial height and stem diameter 5 cm from the ground were 54.8 $\pm$ 0.43 cm and 5.0 $\pm$ 0.06 mm for red maple and 78.4 $\pm$ 1.21 cm and 5.9 $\pm$ 0.08 mm for Washington hawthorn, respectively.

Statistical design. The experimental design was a split-split plot. Main plots were 1) irrigated or 2) not irrigated. Split plots were 1) fertilized at planting or 2) not fertilized. Split-split plots were six soil treatments (described below), completely randomized within each split plot. Main plots (2-m spacing between plots) and split plots (5-m spacing between plots) were replicated twice, and split-split plots were replicated three times (1-m spacing between individual trees). The two species were planted in adjacent rows and the data for each species were analyzed separately.

Soil treatments. Soil treatments were applied at planting as: 1) control (native backfill only); 2) compost (native backfill + yard-waste compost); 3) peat (native backfill + Canadian sphagnum peat); 4 and 5) humate (surface-applied granular humate) at 4) 100 g/tree or 5) 200 g/tree; and 6) liquid humate+, a proprietary liquid mixture of humate, kelp extract, thiamine, and intermediate “metabolites,” applied as a preplant root soak. Compost (Recycling Center, Virginia Tech, Blacksburg, Va.) and peat (Southland sphagnum peat, Greensboro, N.C.) were applied at a rate of 1 amendment : 3 soil (by volume), mixed well before application. Trees in the humate (Earthgreen Products, Dallas, Texas) treatments were backfilled with native soil only (humate was surface applied). Liquid humate+, Roots™ (Roots Inc., New Haven, Conn.), was applied as a 30-min root soak (Roots™ mixed with H₂O at 20 mL·L⁻¹) immediately prior to planting, and holes were backfilled with native soil only. All plots were kept weed-free throughout the experiment with isoxaben {N-[3-(1-ethyl-1-methylpropyl)-2,6-dimethoxybenzamide] + oryzalin (3,5-dinitro-N₄,N₄-dipropylsulfanilamide) and glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine]}.

All trees were well watered at planting and maintained at apparent field capacity for 1 week. Irrigation was withheld thereafter for nonirrigated main plots. Soil moisture levels of irrigated plots were monitored with four resistance blocks (Irrometer, Riverside, Calif.) randomly placed and installed 20 cm deep. Irrigated main plots were irrigated ($\approx$ 1.25) at a mean soil moisture tension of 0.05 MPa using hand irrigation initially, and, later, a

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microirrigation system. Rainfall was monitored with a rain gauge. Ammonium nitrate (34N-0P-0K) was evenly applied to the soil surface of fertilized split plots at planting at a rate of 14.5 g·m⁻² N. The entire experiment received ammonium nitrate at 7.3 g·m⁻² N the following April.

Data collection. Subsequent height and trunk diameter (5 cm from ground level) measurements were taken on 9 Sept. 1995 and 26 Aug. 1996. Tree tops were harvested on 25 Sept. 1996 and dried to a constant mass at 70 °C. In order to determine soil treatment effects on root growth, one main plot replication that received neither irrigation nor fertilizer was randomly selected. This resulted in six trees per soil treatment. All roots for each tree within a 43-cm diameter (centered on stem) and a 30-cm depth were harvested, separated into four diameter classes (0–1 mm, 1–3 mm, 3–5 mm, and 5–10 mm), and dried to a constant mass at 70 °C.

One root system was randomly chosen from each soil treatment, and each diameter class was separated into subsamples of varying amounts for determination of the root length : dry mass relationship. Root length of the subsamples was measured using a combination of computer imaging software (Desk-Scan II; Hewlett Packard, Palo Alto, Calif.) and a computer-image analyzing system (Delta-T SCAN; Delta-T Devices Ltd., Cambridge, England). Subsamples were then dried to a constant mass at 70 °C and weighed. A relationship between dry mass and root length for each diameter class was determined with linear regression (SAS vers. 6.08; SAS Institute, Cary, N.C.) and applied to the respective soil treatment groups for root length determinations. Only root length data are reported.

Height and stem diameter data were analyzed by repeated measures analysis of variance (ANOVA), and planned, single degree of freedom contrasts in the general linear models procedure (GLM) of SAS (SAS vers. 6.08; SAS Institute). Top mass and root length were analyzed with ANOVA and planned, single degree of freedom contrasts in GLM.

Results and Discussion

Top growth—Red maple. Survival was 100%. Irrigation regime affected growth in height ($P = 0.06$, Table 1), but not stem diameter or top dry mass. Mean height was not affected by irrigation at any of three measurement dates (data not shown), although rainfall from May through October was only 60% of normal for 1995 and 80% of normal for 1996. Similar means coupled with a low P value for irrigation effect may be due to different patterns of height changes in individual replications over time. There was no interaction between irrigation and fertilizer regime ($P = 0.25$) or soil treatments ($P = 0.48$). Fertilization at planting had no effect on top growth. Others have reported no response to fertilization at planting (Shoup et al., 1981; Shulte and Whitcomb, 1975; van de Werken, 1981), although Hensley et al. (1988) found an increase for container-grown *Magnolia grandiflora* L.

Table 1. P values from repeated measures ANOVA for effects of irrigation, fertilization, and biostimulants on height and stem diameter and ANOVA for top dry mass for red maple and Washington hawthorn.

Source	Red maple			Washington hawthorn		
	Height ^z (cm)	Stem diam (mm)	Top mass (g)	Height (cm)	Stem diam (mm)	Top mass (g)
Irrigation	0.06 ^y	0.66 ^y	0.13 ^x	0.14 ^y	0.23 ^y	0.23 ^x
Fertilizer	0.30	0.66	0.36	0.84	0.61	0.79
Fertilizer × irrigation	0.25	0.78	0.68	0.86	0.98	0.94
Soil treatment (ST) ^w	0.08	0.07	0.03	0.04	0.39	0.10
ST × irrigation (irr)	0.48	0.02	0.02	0.59	0.52	0.60
ST × fertilizer (fert)	0.73	0.38	0.64	0.55	0.73	0.49
ST × fert × irr	0.90	0.92	0.97	0.45	0.99	0.46

^xTime of measurement was significant at $P \leq 0.05$ for all variables.

^y P values from Wilk's Lambda multivariate tests.

^z P values from F test.

^wCompost, peat, or humate.

Fig. 1. Effects of soil treatments on height of red maple and Washington hawthorn planted 10 May 1995. Single degree of freedom contrast P -values from repeated measures ANOVA are: 1) control vs. others = 0.08 and 0.64; 2) compost vs. biostimulants = 0.08 and 0.004; 3) peat vs. biostimulants = 0.12 and 0.15; 4) control vs. granular humates = 0.09 and 0.20; 5) granular humates vs. liquid humate+ = 0.70 and 0.21; and 6) humate, 100 g vs. humate, 200 g = 0.57 and 0.76 for red maple and Washington hawthorn, respectively.

Fig. 2. Effects of irrigation $\times$ soil treatments on stem diameter of red maple planted 10 May 1995. Single degree of freedom contrast P -values from repeated measures ANOVA are: 1) control vs. others = 0.04 and 0.40; 2) compost vs. biostimulants = 0.008 and 0.46; 3) peat vs. biostimulants = 0.93 and 0.42; 4) control vs. granular humates = 0.005 and 0.89; 5) granular humates vs. liquid humate+ = 0.39 and 0.54; and 6) humate, 100 g vs. humate, 200 g = 0.87 for irrigated and not irrigated treatments, respectively.

We found no delayed 2nd-year response from fertilization at planting as discussed by Harris (1992). The lack of a response in any parameter measured, over two growing seasons, to fertilization at planting with surface-applied ammonium nitrate, a fertilizer with quick response and high salt index (Tisdale and Nelson, 1975), alleviates some concern about salt injury to roots from fertilization at transplanting (Gilman, 1997). Different results may have been obtained with other fertilizer formula-

tions or species. Most growth in height occurred during the second growing season (Fig. 1). Trees with soil treatments grew no more than did controls, and height increase in trees in compost- and peat-amended soil was no greater than that of biostimulant-treated trees (see legend of Fig. 1 for statistics).

Soil treatments interacted with irrigation regime in affecting stem diameter ($P = 0.02$, Table 1). Height of irrigated controls increased more than that of trees in the other treatments

as a group, but nonirrigated controls were similar to others (Fig. 2, see legend). This difference was in part due to the positive effect of irrigation on compost-treated trees, since the latter increased in height more than biostimulant-treated trees only when both groups were irrigated. Change in stem diameters of trees in peat-amended soil was similar to that of biostimulant-treated trees within each irrigation regime, and greater for irrigated controls compared with granular humate-treated trees. All trees had equivalent change when nonirrigated. A similar interaction between irrigation and soil treatment occurred for top dry mass ($P = 0.02$, Table 1; Fig. 3).

Top growth—Washington hawthorn. Survival was 97%. Neither irrigation nor fertilization at planting affected height, stem diameter, or top dry mass (Table 1). As was the case for red maple, soil treatments as a group did not increase height (Fig. 1, see legend). Contrary to data for red maple, however, growth in height was greater for biostimulant-treated trees than for compost-treated trees. Although biostimulant application did not affect height or stem diameter, controls had less top dry mass than others as a group, and less than granular humate-treated trees in particular (Fig. 3, see legend).

Root length—Red maple. Most root length was in the 0–1 mm diameter class. Soil treatments as a group did not increase root length, but the peat treatment was much more effective than the biostimulant treatments (Table 2). Similar results were seen in the 1–3 mm class, although granular humate-treated trees had greater root length than did liquid humate+-treated trees. No contrast was significant for the remaining diameter classes, and totals mirrored the 0–1 mm diameter class. Humate increased root length more at 200 g/tree than at 100 g/tree only in the 1–3 mm root diameter class.

Fig. 3. Effects of irrigation $\times$ soil treatments on top dry mass of red maple and Washington hawthorn planted 10 May 1995. Single degree of freedom contrast P -values from ANOVA are: 1) control vs. others = 0.07, 0.14, and 0.06; 2) compost vs. biostimulants = 0.005, 0.25, and 0.01; 3) peat vs. biostimulants = 0.88, 0.28, and 0.47; 4) control vs. granular humates = 0.03, 0.83, and 0.02; 5) granular humates vs. liquid humate+ = 0.65, 0.04, and 0.21; and 6) humate, 100 g vs. humate, 200 g = 0.70, 0.90, and 0.76 for irrigated red maple, nonirrigated red maple, and Washington hawthorn, respectively.

Table 2. Effects of biostimulants and soil amendments on mean root length (m) by root-diameter class, total root length, and single degree of freedom contrasts for red maple trees; n = 6.

Treatments	Diameter class (mm) ^a				Total (m)
	0-1	1-3	3-5	5-10	
Control	46.1	3.1	1.3	1.5	52.0
Compost	29.3	3.7	1.4	1.4	35.8
Peat	165.8	5.8	1.5	1.2	174.4
Humate, 100 g	57.6	5.2	1.0	1.1	64.9
Humate, 200 g	65.4	3.1	1.0	1.3	70.8
Liquid humate+	35.2	2.3	1.0	1.0	39.3
Source	P				
Treatment	0.0002	0.01	0.24	0.66	0.0002
Control vs. others	0.25	0.27	---	---	0.24
Compost vs. humates	0.30	0.82	---	---	0.32
Peat vs. humates	0.0001	0.009	---	---	0.0001
Control vs. granular humates	0.52	0.28	---	---	0.50
Humates vs. liquid humate+	0.27	0.05	---	---	0.23
Humate, 100 g vs. humate, 200 g	0.77	0.05	---	---	0.83

^aRoots >10 mm in diameter excluded from table.

Table 3. Effects of soil amendments and biostimulants on mean root length (m) by root-diameter class, total root length, and single degree of freedom contrasts for Washington hawthorn trees; n = 6.

Treatments	Diameter class (mm) ^a				Total (m)
	0-1	1-3	3-5	5-10	
Control	55.1	3.3	1.3	1.5	61.2
Compost	21.1	2.4	0.8	1.0	25.3
Peat	88.2	4.0	1.3	0.5	94.9
Humate, 100 g	43.6	2.8	1.3	1.0	48.8
Humate, 200 g	78.5	4.9	1.1	1.6	86.0
Liquid Humate+	59.0	4.4	1.2	1.0	65.6
Source	P				
Treatment	0.0008	0.02	0.48	0.20	0.0007
Control vs. others	0.78	0.47	---	---	0.80
Compost vs. humates	0.002	0.01	---	---	0.001
Peat vs. humates	0.02	0.97	---	---	0.02
Control vs. granular humates	0.63	0.41	---	---	0.63
Humates vs. liquid humate+	0.86	0.41	---	---	0.89
Humate, 100 g vs. humate, 200 g	0.02	0.01	---	---	0.01

^aRoots >10 mm in diameter excluded from table.

Root growth—Washington hawthorn. Most root growth was in the 0–1 mm diameter class, and soil treatments did not increase root length for any diameter class or total root length as a group (Table 3). Peat was again more effective than biostimulants. However, biostimulants were more effective than compost in Washington hawthorn, whereas no differences were evident for red maple. Humate increased root length more when applied at 200 than at 100 g/tree in the 0–1 mm, 1–3 mm, and total root diameter classes.

The results of this study do not support the utility of biostimulant application as an aid to posttransplant establishment of red maple or Washington hawthorn. Soil treatments, on the whole, did not increase height, stem diameter, top dry mass, or root growth of red maple. The use of peat as a soil amendment, however, may increase root length of both species. High root length within rootballs would be desirable for nursery stock, since it may impart resistance to transplant stress. However, rapid root growth outside of the original planting hole is desirable for good plant establishment in landscape soils. We excavated a 43-cm diameter × 30-cm deep area for determination of root length. Although this represents more than a 40% increase in soil volume compared with the original planting hole, comparisons between root length within and beyond the original

hole were not made. High root lengths, therefore, may have been influenced by proliferation of roots within the original backfill. Day et al. (1995) found that soil amendment with peat increased root growth beyond the original planting hole. Although a positive relationship between root length within the harvested soil volume and eventual growth beyond the backfill is assumed, long-term monitoring of tree growth would be required for confirmation. Application of granular humate to Washington hawthorn increased top dry mass, but height, stem diameter, and root length were unaffected by this or other treatments as a group. The lack of effect of biostimulants on root length may be due to above-threshold levels of humic material already in the soil. Humic levels of native soils were not measured in our experiment, however. Different results may have occurred in soils with very low organic matter, such as in many urban soils.

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