Effect of Bioplex™ on Transplant Success of Non-Dormant Red Oak (*Quercus rubra* L.)[1]

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Abstract

Biostimulants are used to reduce the stress associated with non-dormant (summer dug) harvest of field-grown nursery stock; however, the effectiveness of biostimulant treatment is uncertain. This study tested the effects of three application methods of Bioplex™ (a commonly used biostimulant) to container-grown red oak seedlings on whole plant transpirational water use and growth before and after root pruning. Root pruning was used to simulate field harvest; it removed 59% of the seedling's total root surface area. Bioplex™ application by foliar spray, soil drench or a combination of foliar spray and soil drench, significantly reduced whole plant transpirational water use by 15% for three days after application, relative to untreated control seedlings. Root pruning significantly reduced whole plant transpiration, compared to non-root-pruned seedlings, and had a greater effect on transpiration than any Bioplex™ treatment. The previous season's Bioplex treatment had no effect on the spring growth flush following fall root pruning. Root pruning in fall significantly reduced root and total plant dry weights the following spring. Although Bioplex™ applications significantly reduced transpiration for three days after application, there does not seem to be any long-term beneficial effect when used to mediate summer digging transplant stress.

Index words: summer digging, transpiration, *Quercus rubra*, red oak.

Significance to the Nursery Industry

Biostimulants are commonly used to reduce transplant stress, although little information is available on their effectiveness. In our study, Bioplex™ (a type of biostimulant) did decrease short-term transpirational water loss of non-dormant red oak seedlings. Root pruning (used to simulate summer digging) significantly reduced transpiration and had a greater effect on transpiration than any Bioplex™ treatment. Bioplex™ treatment had no beneficial effect on seedling dry weight or growth the following spring. If Bioplex™ is used, the most efficient application method is a foliar spray; foliar application used about half the volume of the soil drench method. However, as indicated on the product label, Bioplex™, foliar application may result in leaf damage in susceptible species. There was no advantage of a combination foliar and soil drench treatment combination over the foliar spray only treatment.

Introduction

In the northern regions of the United States, spring is the primary season to dig balled and burlapped (B&B) nursery stock and consequently the busiest time of year for nurseries. Often frozen soils, soils saturated from spring rains, or early warm weather shorten the digging season. If stock is not lifted by bud break it remains in the field until the fall or spring digging season, which results in lost spring sales. The ability to 'summer dig' would capture lost spring sales by extending the harvest season. For these reasons, nurseries dig some species in summer. Although summer digging procedures vary, the general process includes: saturating the soil, applying an anti-desiccant, digging a larger diameter rootball (compared to that for dormant stock), and acclimating plants under shade and overhead irrigation for several days before shipping.

There is a drastic reduction in the root-to-shoot ratio when nursery stock is dug for B&B shipment; up to a 98% reduction of the original root system (17) or, 91 to 95% of the total root length of *Gleditsia triacanthos* L., *Populus* x *generosa* A. Henry, and *Fraxinus pennsylvanica* Marsh can be left in the field (4). The result is transplant shock.

Transplant shock is the period between transplanting and the resumption of vigorous growth; poor growth encountered during this period is due to internal water deficits (6, 9). Under typical summer conditions, transplant shock can be characterized as transplant induced drought stress. Drought stress causes loss of turgor, reduction in growth, closure of stomata, and a decrease in photosynthesis and metabolic function (8). Therefore, reducing internal water deficits through increased water uptake, rapid root regeneration, or reduced transpirational water loss would reduce internal water stress and increase transplant success. However, reduction in transpirational water loss accomplished through stomatal closure could increase leaf temperature and decrease gas exchange, which would reduce net photosynthesis and winter carbohydrate reserves.

Products known as biostimulants have been marketed specifically as summer transplant aids. Bisostimulants are 'non-nutritional products that may reduce fertilizer use and increase yield and resistance to water and temperature stresses and positively affect plant growth and physiology' (11). Formulas are proprietary but most contain plant hormones, humates, and manure and/or sea kelp extracts (5). Humates have been credited with increasing root growth and water uptake (14, 15). Sea kelp extracts contain high levels of cytokinins, which may be beneficial under stress situations (2).

Russo and Berlyn (11) identified several benefits from biostimulants: increased root and shoot growth, increased resistance to stress, and increased water uptake; any of which would reduce transplant shock. Kelting (7) found biostimulants did not increase B&B red maple (*Acer rubrum* L. 'Fransksred') root growth, but did increase sap flow rates,

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suggesting increased water conductivity. Alternatively, Richardson et al. (15) found biostimulants had a modest effect on plant health, but did not result in greatly improved stress tolerance of treated paper birch (*Betula papyrifera*). A preliminary study done at The Ohio State University showed biostimulants decreased transpiration rates of bur oak seedlings when applied as a foliar spray (Struve and Butcher, unpublished data).

We wanted to study the effects of transplanting non-dormant red oak (*Quercus rubra* L.) seedlings on whole plant water use, transplant survival, and recovery when plants were pre-treated with a biostimulant. Red oak was chosen because it is considered difficult to transplant and is rarely summer dug. It is a deeply rooted species and resists drought stress by avoidance (1). Taxa with coarse root systems have few rapidly regenerating intact root tips remaining after harvest (12). Dormant root pruned red oak mediate transplant shock by reducing leaf area, not by increasing osmotic potential or decreasing photosynthetic gas exchange (on unit leaf area basis), suggesting that internal moisture stress is mediated by drought avoidance, not drought tolerance mechanisms (13). Consequently, it was expected that red oak seedlings with fully developed canopies will not be able to cope with punitive internal water deficits caused by root pruning and thus it would make a good experimental species for studying summer digging. Therefore, the effect of Bioplex™, the biostimulant most commonly used by Ohio nursery managers to mediate putative transplant shock induced by summer-dugging field-grown trees, on whole plant water use before and after root pruning red oak seedlings was studied.

Materials and Methods

Preparation of plant material. Six hundred newly germinated red oak seedlings were purchased from Berg Warner Nursery, Lizton, IN. Acorns were sown in 10.2 cm (4 in) deep flats the previous fall and over-wintered in a walk-in 7C (45F) cooler. In late March, the seedlings were lifted from the flat and root pruned to 6 cm (2.5 in) length. The seedlings were transplanted to 14 cm (5.5 in) square 15 cm (6 in) deep plastic containers (250XL Nursery Supplies, Fairless Hills, PA) treated with Spinout® (Griffin Corp., Valdasta, GA). The medium was Metro Mix 360 (O.M. Scotts and Sons, Marysville, OH). Seedlings remained in the greenhouse from the end of March until late September, 2002. Seedlings were hand watered once or twice daily as needed to prevent soil moisture stress. The seedlings were fertilized weekly with 100 mg/liter 20N–6.3K–8.6P water-soluble fertilizer (20–10–20 Scotts Petelite Fertilizer O.M. Scotts and Sons, Marysville, OH).

Experimental procedures. The experiment began on September 22, 2002, with the random harvest of 20 seedlings. Individual seedling heights, leaf areas, and number of flushes were recorded. The seedlings were then separated into leaves, stems, and roots and placed in a drying oven set at 68C (154F) for two weeks. Roots were severed from the stem mid-way between the transition zone between the green bark and the brown suberized root system. For each seedling, dry weights of the plant parts were recorded. This data collection procedure was followed at each harvest.

Also on September 22, the remaining 440 seedlings had their pots covered with aluminum foil to minimize surface evaporation. The seedlings were placed in sub-irrigation beds constructed specifically for this experiment. The benches were framed with 5 cm × 15.2 cm (2 × 4 in) dimensional lumber and lined with 4 ml poly. Drains were inserted through the poly (2 per bench). The plants rested on 1.2 cm (1/2 in) Styrofoam risers to facilitate drainage. The benches were flooded to a depth of 10.2 cm (4 in) and the seedlings sub-irrigated for one hour. The benches were drained for one hour before taking initial weights of individual seedlings. Weights were recorded daily for the next 3 days (September 22 to 25) using balances (Model No. 12001 Denver Instruments Co., Arvada, CO) connected to a computer allowing individual weights to be logged directly into a spreadsheet. A macro command was written allowing six balances to be used simultaneously. The difference in weights was used to estimate whole plant transpiration. This four-day period was used to establish baseline whole plant transpiration rates for individual seedlings.

On September 25, after the baseline whole plant transpiration rates were determined, seedlings received one of four treatments. The treatments (and number of seedlings per treatment) were: 1) control, no Bioplex™ (N = 140); 2) foliar only application of Bioplex™ (N = 80); 3) soil drench only application of Bioplex™ (N = 80); and 4) a combination of foliar and soil drench applications of Bioplex™ (N = 140). Foliar treatments were applied using a 9.5 liter (2.5 gal) Hudson Leader Plus Sprayer (H.D. Hudson Manufacturing Company, Hastings, MN). Bioplex™ was mixed at a rate of 59 ml (2 oz.) of Bioplex™ per 9.5 liters (2.5 gal) of water. The foliage was sprayed until runoff; we estimated the equivalent of 0.8 ml (0.03 oz) of undiluted Bioplex™ was applied to each seedling. Soil drench applications were delivered using ebb 'n flood benches at a rate of 73 ml (2.5 oz) Bioplex™ per 19 liters (5 gal) of water. Soil drench treated seedlings received an estimated 1.9 ml (0.06 oz) undiluted Bioplex™ (calculated from water holding capacity of medium and Bioplex™ concentration). Those seedlings not receiving a root drench were similarly flooded with tap water.

One hour after the initiation of the root drench treatment seedlings were drained and the initial weights of all pots were recorded (September 25). For the next three days (September 26 to 28), pot weights were recorded daily as described earlier to estimate transpiration rates. After the four-day whole plant transpiration period following Bioplex™ treatment, 20 seedlings from each treatment group were harvested as before.

Also on September 28, seedlings were either root-pruned to simulate transplanting, or left intact. All seedlings in the Bioplex™ foliar and soil drench treatments were root pruned. For the control and combination Bioplex™ foliar and soil drench treatments, half of the seedlings within each group were root pruned, half were left intact. Seedlings were root pruned by removing seedlings from their containers and the roots were severed 5 cm (2 in) below the medium surface with pruning shears. The severed roots and seedlings were then returned to their pots.

To estimate the amount of root surface area and dry weight removed by root pruning, five untreated seedlings were root pruned as prescribed previously on September 28. After root pruning, the medium was carefully washed from the root systems. Severed roots were separated from those intact. For each seedling, the root areas (in cm^{-2}) of severed and intact roots were determined using the WinRHIZO (Regent Instruments Inc., Ste-Foy, Quebec, Canada) system. The percent-

Table 1. **Red oak seedling cumulative water use (g) for four days following Bioplex™ application. Data are for the period of September 25 to 28, 2002.**

Treatment[z]	No. seedlings	Cumulative whole plant transpiration (g)		
		Days after application		
		1	2	3
Control	140	87b[y]	105b	161b
Foliar spray (FS)	80	75a	94a	145a
Soil drench (SD)	80	77a	95a	148a
FS and SD	140	74a	92a	145a

[z]Seedlings given the foliar spray with were sprayed with Bioplex™ until run-off delivering 0.8 ml of undiluted Bioplex™ solution; soil drench treated seedlings received 19 ml undiluted Bioplex™ from a one hour sub-irrigation event; or seedlings were given a combination of soil drench and foliar spray. Control seedling received no Bioplex™ applications.

[y]Means within a column followed by different letters are significantly different from each other at $\alpha = 0.05$ level of significance using Student-Newman-Keuls test.

age of the total root system surface area represented by the severed root area was calculated. Severed and intact root dry weights were determined as before and the percentage of the total root dry weight represented by the severed roots calculated.

After root pruning (September 28) the seedlings were watered using the sub-irrigation benches and drained as described previously and the weights recorded. Individual seedling weights were recorded for the next three days (September 29 to October 1) and used to estimate whole plant transpiration. Eleven days later (October 12), whole plant transpiration was again measured over four days (October 12 to 15) as described previously and 20 seedlings harvested from each treatment group.

The seedlings were then moved to a minimum heat (7C [41F]) polyhouse on October 15, where they were maintained until completion of the first flush in June 2003. At this time 20 seedlings from each treatment group were harvested.

This procedure allowed us to estimate whole plant transpiration and dry weight distribution at the following stages; initial untreated (September 22 to 25), treated but not root pruned (September 25 to 28), treated and root pruned (September 28 to October 1), 11 to 15 days after root pruning (October 12 to 15), and following the spring growth flush (June 2003).

Data analysis. The experiment was a split plot design with three replications. The data were analyzed using the one-way Analysis of Variance (ANOVA) procedure within SPSS for the personal computer (SPSS, Inc. Chicago, IL). The means were separated using the Student-Newman-Keuls at $\alpha = 0.05$ level of significance. Means for whole plant transpiration following root pruning were analyzed using orthogonal contrasts. The contrasts tested were: 1. effect of root pruning without Bioplex™ treatment (Treatment 1 vs 2 [Table 2]); 2. effect of a combination of foliar and soil drench Bioplex™ treatment application without root pruning (Treatment 1 vs 6); 3. effect of root pruning after Bioplex™ treatment (Treatments 3, 4 and 5 vs 2); 4. effect Bioplex™ treatment when applied to foliage and roots (Treatments 3 vs 4); and 5. effect of root pruning with Bioplex™ treatment (Treatments 5 vs 6).

Results and Discussion

On September 22, seedlings averaged 60 cm (24 in) tall with 1335 cm^2 (81 in^2) leaf area. During the three-day pretreatment base-line period (September 22 to 25), average whole plant transpiration was similar for all treatment groups, 51 g (1.8 oz) per day. For three days after Bioplex™ treatment (September 25 to 28; days 1 to 3, Table 1), plants given a foliar spray, soil drench, or foliar spray and soil drench combination, had significantly lower (about 15% [74 vs 87g/day/seedling, combined foliar spray vs control, Table 1]) transpiration than control seedlings.

Root pruning removed 59% of the total root surface area (before root pruning, root surface area averaged 7071 ± 451, after root pruning 4153 ± 508 cm^2) and 14 ± 2.1 g of root dry mass. Whole plant transpiration (September 28 to October

Table 2. **Cumulative water (g) use of red oak seedlings, treated or untreated, with Bioplex™. Seedlings either had been root pruned, to simulate summer digging, or left intact. Bioplex™ was applied four days prior to root pruning. Data are for the period September 28 to October 1, 2002 (days 0 to 3) and October 11 to 14 (days 14–17).**

Treatment	Bioplex™ treatment[z]	Root pruned	Cumulative whole plant transpiration (g)				
			Days after root pruning				
			0–1	0–2	0–3	14–15	14–17
1	None	No	114	149	246	43	194
2	None	Yes	55	78	135	31	136
3	Foliar spray (FS)	Yes	62	85	144	28	127
4	Soil drench (SD)	Yes	51	68	122	28	124
5	FS and SD	Yes	56	78	135	28	144
6	FS and SD	No	106	143	242	41	196
Contrast 1: Treatment 1 vs 2			0.001	0.001	0.001	0.001	0.001
Contrast 2: Treatment 1 vs 6			0.117	0.325	0.681	0.327	0.837
Contrast 3: Treatment 3, 4 and 5 vs 2			0.834	0.952	0.898	0.101	0.531
Contrast 4: Treatment 3 vs 4			0.060	0.005	0.025	0.999	0.753
Contrast 5: Treatment 5 vs 6			0.001	0.001	0.001	0.001	0.001

[z]Seedlings given the foliar spray were sprayed with Bioplex™ until run-off delivering 0.8 ml of undiluted Bioplex™ solution; soil drench treated seedlings received 19 ml undiluted Bioplex™ from a one hour sub-irrigation event; or seedlings were given a combination of soil drench and foliar spray. Control seedlings received no Bioplex™ applications.

Table 3. Red oak growth and dry weight distribution in June 2003 (after the completion of the spring growth flush) after root pruning to simulated summer digging the previous September, as affected by Bioplex™ treatment.

Treatment no.	Treatment combination: Bioplex™ treatment[z]	Treatment combination: Root pruned	Total height (cm)	Spring growth flush (cm)	Leaf area (cm^2)	Leaf no.	Dry weight (g) leaf	Dry weight (g) root	Dry weight (g) stem
1	None	No	90	32	1770	46	8.8	23.9	15.1
2	None	Yes	87	28	1589	55	7.6	17.1	14.7
3	Foliar spray	Yes	91	33	1426	47	6.9	16.9	13.9
4	Soil drench	Yes	81	22	1567	54	7.1	16.7	12.4
5	Foliar spray and Soil drench	Yes	86	24	1572	51	7.8	16.3	12.6
6	Foliar spray and Soil drench	No	87	29	1907	49	9.4	23.9	13.8
Contrast 1: Treatment 1 vs 2			0.415	0.189	0.118	0.047	0.157	0.001	0.662
Contrast 2: Treatment 1 vs 6			0.490	0.421	0.243	0.418	0.472	0.991	0.263
Contrast 3: Treatment 3, 4 and 5 vs 2			0.749	0.386	0.487	0.199	0.596	0.788	0.089
Contrast 4: Treatment 3 vs 4			0.003	0.001	0.236	0.045	0.804	0.918	0.206
Contrast 5: Treatment 5 vs 6			0.799	0.063	0.006	0.773	0.074	0.001	0.304

[z]Seedlings given the foliar spray were sprayed with Bioplex™ until run-off delivering 0.8 ml of undiluted Bioplex™ solution; soil drench treated seedlings received 19 ml undiluted Bioplex™ from a one hour sub-irrigation event; or seedlings were given a combination of soil drench and foliar spray. Control seedlings received no Bioplex™ applications nor root pruning.

15) of root-pruned seedlings was less than that of intact seedling by 52, 48, 45, 27 and 30% at 1, 2, 3, 15, and 17 days after root pruning, respectively (Table 2, Contrast 1). There were no significant differences in whole plant transpiration between intact seedlings and intact seedlings given a combination of foliar and soil drench Bioplex™ (Table 2, Contrast 2). For root-pruned seedlings, the no-Bioplex™ treatment did not reduce transpiration compared with root pruned seedlings treated with Bioplex™ (Table 2, Contrast 3). Comparing application methods, foliar spray vs soil drench showed the soil drench treatment group reduced whole plant transpiration more than the foliar spray treatment group for 2 and 3 days after root pruning, with no reduction in whole plant transpiration 1, 15, or 17 days after root pruning (Table 2, Contrast 4). Whole plant transpiration of root pruned seedlings treated with a foliar spray and soil drench was 53, 55, 56, 68, and 73% of intact seedlings treat with a foliar spray and soil drench at 1, 2, 3, 15, and 17 days after root pruning (Table 2, Contrast 5).

In the spring growth flush following root pruning the previous fall, root-pruned plants had significantly more leaves, but less root dry weight than unpruned seedlings (Table 3, Contrast 1). There were no statistical differences between unpruned seedlings treated with a combination of foliar and soil drench Bioplex™ treatment and those not treated with Bioplex™ (Table 3, Contrast 2), nor were there any differences between root-pruned Bioplex™ treated seedlings and untreated root-pruned seedlings (Table 3, Contrast 3). Total height and height of spring 2003 growth flush were greater for seedlings given a Bioplex™ foliar spray than the soil drench, while soil drenched seedlings had more leaves (Table 3, Contrast 4). Leaf area and root dry weight were greater for the intact seedlings receiving the foliar spray and soil drench treatment compared to root pruned seedlings also receiving the foliar spray and soil drench treatment (Table 3, Contrast 5).

The objective of this research was to quantify the effects of transplanting (root loss) and biostimulant application on whole plant water use and growth of northern red oak. Bioplex™ treatment (regardless of application method) caused an immediate, but short-lived reduction in whole plant transpiration (Table 1). The greatest reduction was only 15%. In June 2003 following root pruning in September 2002, growth was similar for all treatment groups, except root pruning increased leaf number and decreased root dry weight. No benefit of biostimulant application was observed in the following year's growth.

In this study, whole plant transpiration rates were temporarily decreased by biostimulant application, unlike previous studies (3, 7, 11, 14, 15). The foliar application of Bioplex™ delivered 42% less active ingredient than the soil drench treatment, but caused a similar short-term reduction in whole plant transpiration, suggesting a leaf mediated mechanism of action. Stomatal closure reduces transpirational water loss (8) and may be responsible for the differences seen here. Due to the reduction in photosynthesis, also associated with closed stomates, this is not a preferred strategy for replenishing lost resources associated with harvest, and not likely to promote establishment. Response to the application only lasted three days, after which whole plant transpiration rates returned to that of untreated seedlings. Re-application may be necessary to maintain reduced transpiration.

Root pruning caused a long-term reduction in transpiration suggesting the development of internal water deficits following transplant (6, 9). Even with this large difference in water use of root pruned and non-root pruned seedlings, moisture stress symptoms were not observed. There was no early fall color development, defoliation, or die back, nor did any seedlings die. This may be due to the less severe root pruning treatment used in the experiment. The total root surface area was reduced root 59%, while field digging results in greater losses (4, 16). The differences observed in transpiration rates of root pruned and intact seedlings diminished over time; the rate of recovery was similar for biostimulant treated and non-treated seedlings. Whole plant transpiration of root pruned seedlings had not completely recovered after eighteen days.

Root pruned seedlings developed more and smaller leaves compared to intact seedlings, similar to findings by Struve and Jolly (13). Reduction in leaf area is a drought avoidance mechanism that can prevent or reduce internal water deficits. Root pruning accounted for significant differences in dry root weight of root pruned and non-root pruned seedlings in June following late-season root pruning. However, in contrast to other studies, biostimulant application did not increase root dry weight of root pruned seedlings (11, 14, 15). Red oak was chosen due to its classification as a hard-to-transplant species with drought avoiding stress mechanisms. This study suggests there are no consequences, besides smaller leaves, associated with root pruning red oak seedlings. Further, under the conditions of this study, Bioplex™ did not reduce transplant shock in red oak seedlings. However, the benign environment of the greenhouse, along with adequate watering following root pruning, may have prevented mortality. Therefore, the stress experienced by the seedlings may not have been sufficiently great to reveal any benefits from the biostimulant Bioplex™.

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