Auxin Concentration Affects Adventitious Rooting of Mound Layered Caddo Sugar Maple (*Acer saccharum*) and Shantung Maple (*Acer truncatum*)[1]

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Abstract

Stump sprouts of stooled caddo sugar maple (*Acer saccharum* Marsh. subsp. *saccharum*) (caddo maple) and shantung maple (*A. truncatum* Bunge) were propagated by mound layering. In early summer, shoot bases were wounded and treated with 0, 10,000, 15,000, or 20,000 ppm (0, 1.0, 1.5, or 2.0%) indole-3-butyric acid (IBA) dissolved in 50% ethanol or 0, 5,000, or 10,000 ppm (0, 0.5, or 1.0%) of the potassium (K) salt of IBA (K-IBA) dissolved in water. Shoot bases were covered with a commercial growing substrate containing bark, Sphagnum peat moss, and perlite and retained by plastic rings 38 cm (15 in) in height. Rooted shoots were harvested in fall or late winter. Auxin concentration influenced rooting of caddo maple and shantung maple mound-layered shoots. Rooting peaked at 15,000 ppm (1.5%) IBA for both caddo maple (71%) and shantung maple (34%). Mean root number for caddo maple, but not shantung maple, increased as IBA concentration increased. Differences in mean root length were not significant. Results suggest caddo maple can be successfully propagated by mound layering. For shantung maple propagation, mound layering was less successful, so alternatives such as stem cuttings should be considered.

Index words: IBA, K-IBA, indole-3-butyric acid, mound layering, stooling.

Species used in this study: caddo sugar maple (*Acer saccharum* Marsh. subsp. *saccharum*); shantung maple (*Acer truncatum* Bunge).

Significance to the Horticulture Industry

Stooled shoots of caddo sugar maple (*Acer saccharum* Marsh. subsp. *saccharum*) (caddo maple) and shantung maple (*A. truncatum* Bunge) were successfully rooted by mound layering. Best rooting in both species occurred when the basal portions of shoots were heavily wounded by scraping one side of each stem with a grafting knife to expose its xylem at an internode and treated with 15,000 ppm (1.5%) indole-3-butyric acid (IBA) dissolved in 50% aqueous ethanol (EtOH) in early summer. After treatment, shoot bases were covered with moist container growing substrate until roots developed and shoots became dormant in fall. Because caddo maple rooted well (71%), interested growers should consider propagating this species by mound layering. Seed of caddo maple are likely hybridized with surrounding non-caddo maples, budding of caddo maple can be difficult, and stem cuttings root poorly. Therefore, mound layering may be the most efficient method to propagate caddo maple on their own roots. Shantung maples rooted poorly (34%), and growers are advised to seek alternative methods of vegetatively propagating this species, such as stem cuttings or budding.

Introduction

The ornamental landscape industry seeks plants with attractive, dependable growth characteristics and tolerance to environmental stresses. Caddo maple and shantung maple both offer desirable traits. Caddo maple originated from a disjunct population of sugar maples (*Acer saccharum* Marsh.) native to central Oklahoma and is known for its tolerance to drought, heat, wind, and alkaline soil (Griffin 2014, Pair 1994b, Simpson and Hipp 1993). Shantung maples are native to northern China, Russia, Japan, and Korea (Dirr 2009; Pair 1986). They also perform well in hot, dry environments and possess excellent pest resistance (Griffin 2014, Pair 1986, Pair et al. 1996). Maturing at 6 to 8 m (20 to 25 ft) (Dirr 2009), shantung maples fit nicely into urban landscapes.

Both species are relatively difficult to propagate asexually, which hinders the introduction and availability of improved cultivars. Currently, cultivars are propagated by bench grafting or budding (LeDuc and Pair 2000, Pair 1994a, Pair et al. 1996, Vertrees 1978), but these methods are labor intensive. Furthermore, questions remain regarding potentially compromised drought and pH adaptability due to rootstock selection (Le Duc and Pair 2000).

Alternatives to grafting include micropropagation, vegetative cuttings, and layering of stock plants. Vegetative cuttings produce mixed results. Podaras and Bassuk (1996) report rooting percentages of shantung maple as high as 88% from softwood stem cuttings that were banded, etiolated, and treated with 5,000 ppm (0.5%) indole-3-butyric acid (IBA). Without banding or etiolating, Pair (1986) achieved 62% overall rooting of semi-hardwood stem cuttings treated with 0 to 5,000 ppm (0 to 0.5%) IBA. In recent work using a range of cutting dates, IBA concentrations, and solvents, stem cuttings of shantung maple failed to root greater than 55% (Brock 2014). Additional information on auxin products registered for plant propagation and their use was reported by Boyer et al. (2013).

Although sugar maples in general have rooted successfully in some studies, specific research with caddo maple has yielded poor results. Morsink (1971) reported 80 to 89% rooting of softwood sugar maple stump sprouts collected and prepared as 35 to 65 cm (14 to 26 in) cuttings without IBA application. However, when Alsup (2001) attempted to root

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shorter [7 to 20 cm (3 to 8 in)] stem cuttings of caddo maples, only 30% of softwood stem cuttings rooted using 5,000 to 15,000 ppm (0.5 to 1.5%) IBA quick dip.

When necessary, mound layering can be employed for species that root poorly from stem cuttings. Examples include apple (*Malus* Mill.) (Howard 1977), oaks [swamp white oak (*Quercus bicolor* Willd.) and bur oak (*Q. macrocarpa* Michx.)] (Amissah and Bassuk 2005), and Chinese pistache (*Pistacia chinensis* Bunge) (Dunn and Cole 1995). Rupp et al. (2013) achieved 16% rooting of mound-layered shoots of bigtooth maple [*A. saccharum* subsp. *grandidentatum* (Nutt.) Desmarais] using 4,000 ppm (0.4%) IBA with 2,000 ppm (0.2%) 1-naphthalene acetic acid (NAA). Rooting was increased to 87% when auxin treatment was combined with girdling of the base of stems. Alsup (2001) attempted to propagate caddo maple by mound layering shoots from 2-year-old seedlings. Rooting was improved when shoots were wounded at the base and 5,000 ppm (0.5%) IBA was applied to the wound. Alsup suggested that future studies establish stool beds from larger stock plants. The objective of the current study was to determine the optimal IBA concentrations for rooting mound layered caddo maple and shantung maple shoots.

Materials and Methods

Stock plants were 15 to 20-year-old field grown caddo maple and shantung maple trees of seedling origin (non-clonal) growing in a Canadian-Waldeck fine sandy loam soil at the Kansas State University John C. Pair Horticulture Center near Haysville, KS. Trees were stooled at 10 to 15 cm (4 to 6 in) above the soil surface in spring 2010. Stump diameters were 7 to 13 cm (3 to 5 in). In subsequent years, all shoots were removed from the stock plant at their point of attachment prior to bud-break. All trees received 14 g (0.5 oz) of nitrogen (N) from urea (46N-0P-0K) in April 2011, 2012, and 2013.

Each spring, when shoots reached 50 to 100 cm (20 to 39 in) in height and were actively growing, leaves were stripped from the basal 35 cm (14 in) of shoots, and stems less than 0.5 cm (0.2 in) basal diameter were removed. Most stock plants produced 15 to 20 shoots. Treatments were applied within 13 d of stock plant preparation. In 2013 only, due to reduced shoot elongation, leaves of shantung maple shoots were stripped from the basal 20 cm (8 in).

In 2012, stems of both species were wounded between the second and third basal nodes with two 5 cm (2 in) shallow longitudinal cuts through the phloem, on opposite sides of the stem. Three rates of K-IBA (Sigma-Aldrich, St. Louis, MO) dissolved in reverse osmosis water at 0, 5,000, and 10,000 ppm (0, 0.5, and 1.0%) were immediately applied to the entire circumference of the shoot at the wound using a clean foam paint brush. At harvest, it was observed that these lightly-wounded shoots had completely sealed over with little sign of callus development. Therefore, in 2013, heavy wounding was achieved by scraping one side of each stem with a grafting knife to expose its xylem at an internode 5 to 10 cm (2 to 4 in) from its point of attachment to the stump, leaving a wound 3 to 4 cm (1.2 to 1.6 in) in length and 0.25 to 0.5 cm (0.1 to 0.2 in) in width. Auxin was immediately applied in the same manner as 2012. In 2013, auxin treatments were four rates of the free acid of IBA (≥ 99.0%, Sigma-Aldrich, St. Louis, MO) dissolved in EtOH:water (1:1 v/v) at 0, 10,000, 15,000, and 20,000 ppm (0, 1.0, 1.5, and 2.0%). In both years, each auxin treatment was assigned randomly to five shoots (subsamples) on each stump (block). Due to a shortage of shoots in 2013, the 15,000 ppm (1.5%) IBA treatment was omitted from one shantung maple and five caddo maple stumps. Treatments were applied to caddo maple on June 27, 2012, and June 25, 2013, and to shantung maple on June 8, 2012, and July 10, 2013.

After allowing the auxin to adhere to the shoot surface (5 min), substrate retention rings were placed around treated stock plants. Inverted #15S [46 L (12 gal); 38 cm (15 in) in height], nursery pots (Poly-Tainer Inc., Simi Valley, CA) with their bottoms cut out were used to retain the substrate on shantung maples in 2012. For caddo maples in 2012 and both species in 2013, substrate retention rings were 160 × 38 cm (60 by 15 in) sections of perforated root wrap (Root-Builder II Expandable Container, RootMaker Products Co., LLC, Huntsville, AL) secured with cable ties to form rings with 50 cm (20 in) diameters and 75 L (20 gal) capacity. The outside of all rings was painted white to reduce heating due to light absorption.

Once the substrate retention rings were placed around the auxin treated shoots, they were filled with a commercial container production substrate containing bark, Sphagnum peat moss, and perlite (Metro-Mix 900, Sun Gro Horticulture, Agawam, MA) to cover the portion of the shoots which had been stripped of leaves. All wound sites were covered by at least 10 cm (4 in) of substrate.

Supplemental irrigation was applied as needed by overhead irrigation and hand watering to maintain moist conditions in the rooting substrate and in the soil around stock plant roots. Rooting substrate temperature was monitored with five thermometers randomly assigned to five mound-layered plants during 2013. They were placed vertically in the top 20 cm (8 in) of the growing media, 8 cm (3 in) from the south facing (warmest) side of each ring.

For the first experiment, shoots of both species were harvested on November 19, 2012, 21 and 24 weeks after treating the caddo maple and shantung maples, respectively. For the second experiment, shoots of both species were harvested on March 12, 2014, 37 and 35 weeks after treating the caddo maple and shantung maples, respectively in 2013. Substrate retention rings were removed and rooting substrate was teased gently away from the stock plants. Rooted shoots were severed from the stock plants at their point of origin. Root number and total length of primary roots were measured for each rooted shoot.

The experimental design was a randomized complete block design with 3 (2012) or 4 (2013) auxin concentrations and 5 shoots (subsamples) per auxin concentration per stock plant (block). There were 19 stock plants (blocks) of caddo maple both years. Of the shantung maples, there were 19 stock plants (blocks) in 2012, but 20 stock plants in 2013. Data were subjected to analysis of variance using the PROC GLM statement of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Where appropriate, data were also subjected to regression analysis.

Results and Discussion

Caddo maples rooted successfully by mound layering in both 2012 and 2013, while shantung maples only rooted in 2013. Stock plants remained healthy and shoots grew vigorously throughout both seasons. Substrate temperatures reached 29C (85F) during afternoons on days when air

Table 1. **Percent rooting, mean root number, and mean primary root length of mound layered caddo maple (*Acer saccharum* subsp. *saccharum*) shoots treated June 27, 2012, with potassium (K) salt of indole-3-butyric acid (K-IBA) dissolved in water.[z]**

K-IBA (ppm)	Rooting[y] (%)	Root no.	Root length (cm)
0	4.3	1.5	15.0
5,000	12.6	2.8	16.1
10,000	37.0	7.8	17.6
P ≤	0.0001	0.08	0.66
Linear regression[x]	**	NS	NS

[z]Layered shoots were wounded and the base covered with commercial potting substrate.

[y]N = 89, 89, and 90 shoots for 0; 5,000; and 10,000 ppm, respectively.

[x]Nonsignificant (NS) at $P \leq 0.05$ or (**) Significant at $P \leq 0.01$.

temperatures reached 38C (100F) (data not shown). This temperature is unlikely to inhibit rooting.

Caddo maples. Increasing K-IBA and IBA concentration increased rooting of caddo maple shoots. In 2012, percent rooting rose linearly to 37% at 10,000 ppm (1.0%) K-IBA (Table 1). Root number (4.0) and mean root length per rooted shoot [16.2 cm (6.4 in)] were unaffected ($P \leq 0.08$ and $P \leq 0.66$, respectively) by K-IBA concentration. In 2013, percent rooting responded quadratically and peaked at 15,000 ppm (1.5%) IBA reaching 71.1% (Table 2). Rooting decreased to 54% when IBA was increased to 20,000 ppm (2.0%). Mean root number per rooted shoot also increased with higher concentrations of IBA, reaching 29.5 roots when treated with 20,000 ppm (2.0%). Mean root length, however, was unaffected ($P \leq 0.72$) by increasing IBA concentration [12.2 cm (4.8 in)].

These results agree with work by others. Rupp et al. (2013) and Alsup (2001) demonstrated that IBA treatments could improve rooting of layered sugar maple subspecies, but optimal rates were not established. Based on results from the current study, 15,000 ppm (1.5%) IBA promotes best rooting of mound-layered caddo maple shoots. Further research may improve rooting of individual cultivars by investigating more specific rate increments within the 10,000 to 20,000 ppm (1.0 to 2.0%) range.

Among the stem cutting and mound layering propagation methods investigated by Alsup (2001), caddo maple rooted best (52.9%) from shoots which were wounded and treated with IBA before mound layering. The current study has improved the success of caddo maple propagation by mound layering (71.1% rooting), making it now one of the most productive methods of obtaining caddo maple clones on their own root systems.

The positive relationship between IBA concentration and root number agrees with the current understanding that IBA induces root initiation (Taiz and Zeiger 2006). Although IBA concentrations in this study failed to show the upper limit of the response of mean root number to IBA concentration, the results do suggest an upper limit to rooting percentage. Additionally, 29.5 roots per rooted shoot should be sufficient to support the shoots after harvest.

The similarity of mean root length between the two experiments suggests that most first season root elongation from layered shoots occurs by late autumn. Although shoots treated in 2013 remained on stock plants 4 months longer than those treated in 2012, their mean root length was no longer than the mean root length of shoots of 2012.

Shantung maples. All shoots of shantung maple failed to root in 2012. Shoots appeared healthy throughout the growing season. At harvest it was observed that shoots had sealed over wound sites without producing roots.

In 2013, percent rooting (32.4%) peaked at 15,000 ppm (1.5%) IBA (Table 3).There was a strong quadratic rooting response to increasing IBA concentration and percent rooting decreased to 15.9% when shoots were treated with 20,000 ppm (2.0%). Mean root number (5.9) and mean root length per rooted shoot [11.4 cm (4.5 in)] were unaffected ($P \leq 0.32$ and $P \leq 0.36$, respectively) by IBA concentration.

Table 2. **Percent rooting, mean root number, and mean primary root length of mound layered caddo maple (*Acer saccharum* subsp. *saccharum*) shoots treated June 25, 2013, with the free acid of indole-3-butyric acid (IBA) dissolved in ethanol (EtOH):water (1:1 v/v).[z]**

IBA (ppm)	Rooting[y] (%)	Root no.	Root length (cm)
0	10.0	4.3	10.8
10,000	44.4	10.2	13.1
15,000	71.1	19.2	11.9
20,000	54.1	29.5	12.9
P ≤	0.0001	0.02	0.72
Linear[x]	**	**	NS
Quadratic	*	NS	NS

[z]Layered shoots were wounded and backfilled with commercial potting substrate.

[y]N = 89, 91, 67, and 90 shoots for 0; 10,000; 15,000; and 20,000 ppm, respectively.

[x]Nonsignificant (NS) at $P \leq 0.05$, (*) Significant at $P \leq 0.05$, or (**) Significant at $P \leq 0.01$.

Table 3. **Percent rooting, mean root number, and mean primary root length of mound layered shantung maple (*Acer truncatum*) shoots treated July 10, 2013 with the free acid of indole-3-butyric acid (IBA) dissolved in 1 ethanol (EtOH): 1 water (v/v).[z]**

IBA (ppm)	Rooting[y] (%)	Root no.	Root length (cm)
0	0.0	—	—
10,000	19.0	6.7	9.5
15,000	32.4	4.3	11.7
20,000	15.9	6.7	13.0
P ≤	0.0001	0.32	0.36
Linear[x]	**	NS	NS
Quadratic	**	NS	NS

[z]Layered shoots were wounded and backfilled with commercial potting substrate.

[y]N = 100, 100, 94, and 100 shoots for 0; 10,000; 15,000; and 20,000 ppm, respectively.

[x]Nonsignificant (NS) at $P \leq 0.05$ or (**) Significant at $P \leq 0.01$.

The effect of IBA concentration upon rooting of shantung maple parallels the response observed in caddo maple. Based on this study, mound-layered shantung maple shoots root best when treated with 15,000 ppm (1.5%) IBA. As with caddo maple, further research may optimize this IBA concentration for specific cultivars by evaluating smaller increment rates from 10,000 to 20,000 ppm (1.0 to 2.0%). This high IBA rate is not surprising for a species notoriously difficult to root. However, the high IBA rate optimal for mound layering does seem odd when compared to a recent study which showed that rooting of shantung maple stem cuttings has a negative relationship to increasing concentrations of IBA (Brock 2014). The difference in size and developmental state of propagation material in the two studies likely accounts for the different responses. Semi-hardwood stem cuttings have a smaller diameter and are more tender than the lignified bases of shoots used for mound layering.

Although the current study has demonstrated the feasibility of rooting shantung maples by mound layering, the efficiency of this method remains low. Currently, stem cuttings are the most efficient method of propagating shantung maple cultivars on their own root systems. Future research may improve the efficiency of both cutting propagation and mound layering.

The improved rooting of shoots of both species in 2013 compared to 2012 cannot be attributed to any single factor because adjustments were made to the experimental design in 2013 and other changes occurred. These differences included a new style of substrate retention rings for the shantung maples, heavier wounding of shoots, and replacing water with EtOH:water (1:1 v/v) as an auxin solvent. Other differences include yearly variation in the developmental stage at which treatments were applied and the age of the stock plants, which were potentially rejuvenated by successive seasons of stooling. Of these factors, heavier wounding and the IBA solvent probably played the biggest role. Heavy wounding likely interfered more with carbohydrate translocation than the light wounding did in 2012. This principle is often applied to air layering. Using EtOH:water should have improved penetration of IBA into shoot tissue. When applied to stem cuttings of shantung maple, recent work (Brock 2014) found that EtOH:water slightly improved rooting (48.6%) compared to treating cuttings with only water (37.1%).

Caddo maple and shantung maples both rooted best in mound-layering treatments when treated with 15,000 ppm (1.5%) IBA. Using EtOH:water as an IBA solvent and heavily wounding the base of stems likely enhanced rooting. Growers may now propagate caddo maple on its own root system by mound layering. For shantung maple propagation, stem cuttings remain the best alternative to grafting.

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