Rooting Response of Stem Cuttings of Shantung Maple (*Acer truncatum*) to Time of Year, Cutting Position, and Auxin Concentration, Formulation, and Solvent¹

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Abstract

The influence of time of year, cutting position, and auxin concentration, formulation, and solvent on rooting of stem cuttings of shantung maple (*Acer truncatum* Bunge) was investigated in seven experiments. Softwood, semi-hardwood, second-flush softwood, and hardwood stem-tip cuttings were compared. Semi-hardwood cuttings [15 cm (6 in) in length] rooted best (55%). Auxin treatments [indole-3-butyric acid (IBA) or the potassium (K) salt of IBA (K-IBA)] ranged from 0 to 15,000 ppm (0 to 1.5%). Generally, rooting percentage decreased as auxin concentration increased. Cutting position (terminal or subterminal), auxin formulation (liquid or powder), and auxin solvent [water or ethanol:water (1:1 v/v)] did not affect percent rooting. Mean root number and mean root length were unaffected by all treatments. Results suggest shantung maple roots best from semi-hardwood cuttings treated with auxin at concentrations of 0 or 2,500 ppm (0.25%).

Index words: IBA, indole-3-butyric acid, K-IBA, purpleblow maple, vegetative propagation.

Species used in this study: shantung maple (Acer truncatum Bunge).

Significance to the Horticulture Industry

Shantung maples possess the heat and drought tolerance necessary to thrive in landscapes throughout the southern Great Plains and is hardy to USDA Hardiness Zone 4 (Dirr 2009). Improving cutting propagation techniques could increase the availability of this species. This study investigated factors of shantung maple cutting propagation. Stem-tip cuttings rooted best when collected as 15 cm (6 in) semihardwood cuttings with leaves fully expanded and stems lignified. Treating cuttings with a quick-dip in indole-3butyric acid (IBA) dissolved in 50% aqueous ethanol or the potassium (K) salt of IBA (K-IBA) dissolved in water did not improve rooting and in one experiment significantly decreased rooting at concentrations > 2500 ppm (0.25%). Cutting position within the original stock plant shoot (terminal or subterminal), auxin formulation (liquid or powder), and auxin solvent type (water or ethanol) did not affect rooting percentage, average root number, or average root length. Growers wanting to propagate shantung maple by cuttings should select semi-hardwood cuttings and should not apply IBA or K-IBA at concentrations > 2,500 ppm (0.25%). For further information regarding auxin products registered for plant propagation and their use, see Boyer et al. (2013).

Introduction

Several characteristics of shantung maple (*Acer truncatum* Bunge), also referred to as purpleblow maple, suit it well for landscapes in the southern Great Plains. Maturing at 6 to 8 m (20 to 25 ft) in height (Dirr 2009), this drought tolerant species displays purple-tinged new growth and fall colors ranging from yellow to orange to red. Currently, growers

propagate the few existing cultivars by labor-intensive grafting techniques. An efficient method of propagating shantung maples by stem cuttings could improve the availability and popularity of this promising species.

Previous attempts to propagate shantung maple by stem cuttings have produced varied results. Vertrees (1978) noted anecdotally that cuttings of shantung maple collected during late June in Oregon failed to root when treated with 8,000 ppm (0.8%) IBA in talc powder (IBA/talc). The author suggested that a 1,000 ppm (0.1%) IBA quick-dip be used and noted that collecting cuttings at a proper developmental stage is critical. Podaras and Bassuk (1996) treated softwood stem cuttings from a 4-year-old greenhouse-grown shantung maple with a 20-s dip in solutions of 0, 1,000, 5,000, and 10,000 ppm (0, 0.1, 0.5, and 1.0%) IBA dissolved in ethanol:water (1:1 v/v). They obtained the best rooting (51 to 56%) at 0 and 1,000 ppm (0 and 0.1%) IBA, respectively. Etiolating and banding cuttings before harvest increased rooting to 88% at a 5,000 ppm (0.5%) IBA treatment. Softwood cuttings collected from a mature field specimen rooted poorly ($\leq 21\%$).

Pair (1986) reported good rooting of semi-hardwood shoot tip cuttings from 3-year-old shantung maple seedlings [75% rooting after a quick (5-s) dip in 1000 ppm (0.1%) IBA; solvent not specified]. Rooting of subterminal cuttings (the cutting immediately proximal to the shoot tip cutting) reached 85% at 5000 ppm (0.5%) IBA. This data suggests that stock plant maturity plays an important role in rooting of stem cuttings. Unfortunately, this experiment lacked sufficient repetition to provide confidence in the results. The objective of this study was to build upon Pair's (1986) work using a statistically stronger experimental design to investigate the role of stock plant growth stage, cutting position, and auxin concentration, formulation, and solvent on rooting of stem cuttings of shantung maple.

Materials and Methods

General conditions. Stock plants were 19 mature halfsibling shantung maple specimens of seedling origin (nonclonal) \approx 20 years old, growing at the Kansas State University John C. Pair Horticulture Center near Haysville, KS. To encourage vigorous epicormic shoot growth and increase

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the quantity of stem cuttings, ten of the trees were topped at 1.5 m (4.9 ft) in spring 2010. In spring 2013, nine more trees were topped at 1.5 m (4.9 ft) to increase available cutting material. All cuttings in this study were collected from the original ten stock plants except the second-flush softwood cuttings of 2013, which were collected from only the nine recently topped stock plants of 2013. All trees received 14 g (0.5 oz) of nitrogen (N) from urea (46N-0P-0K) applied in April each year cuttings were taken.

For each experiment in this study, cuttings were categorized as softwood, semi-hardwood, second-flush softwood, or hardwood. Softwood cuttings were collected from activelgrowing shoots before leaves at the shoot apex fully expanded and stems lignified. Semi-hardwood cuttings were collected after shoot elongation ceased, leaves near the shoot apex reached their full size, and stems were lignified. Second-flush softwood cuttings were identical to softwood cuttings except they were collected from the flush of growth that occurred in late summer. Hardwood cuttings were collected in late winter from dormant shoots. All cuttings were collected from the most recent season's growth.

During mornings of cutting collection days, 20–30 cm (8–12 in) terminal stem cuttings were collected from stock plants. Each cutting was trimmed to 15 cm (6 in) from the terminal bud. In addition to the second-flush softwood terminal cuttings of 2013, subterminal cuttings were also collected. These 15 cm (6 in) subterminal cuttings were taken directly proximal to the terminal cutting beginning with the next node. Leaves were stripped from the basal half of each cutting before treatments were applied.

Cuttings were thoroughly mixed before applying treatments to blend any genetic variation of stock plant propensity for rooting. However, in the second-flush softwood cuttings of 2013, terminal stem cuttings from each stock plant were assigned to a specific block to ensure genetic uniformity within each block.

Cuttings for all experiments were inserted to a depth of 5 cm (2.0 in) in 38-cell trays with individual cells [6.4 cm diam by 12.7 cm deep (2.5 in diam by 5 in deep)] (X-38ST, Land-mark Plastic, Akron, OH) filled with moist perlite:sphagnum peat moss (3:1 v/v) substrate. Trays of cuttings received intermittent mist during natural daylight hours in a polycarbonate greenhouse covered with 63% shadecloth (WS63, DeWitt Co., Sikeston, MO). Mist on softwood and semi-hardwood cuttings operated for 6 s every 7 min, whereas mist on hardwood cuttings operated for 10 s every 30 min. No supplemental light was provided. Temperature in the greenhouse was set to 21/16C (70/60F) (day/night) and controlled using an evaporative cooling system.

Treatments in this study consisted of auxin concentration [0, 2,500, 5,000, 10,000, and 15,000 ppm (0, 0.25, 0.5, 1.0, and 1.5%)], auxin formulation [liquid or talc powder (Rhizopon AA #1, #2, and #3, Rhizopon B.V., Hazerswoude-Rijndijk, Netherlands], auxin solvent [water or ethanol:water (1:1 v/v)], and cutting position (terminal or subterminal). K-IBA (Sigma-Aldrich, St. Louis, MO) was dissolved in reverse osmosis water (treatment K-IBA/H₂O) whereas the free acid of IBA (\geq 99.0%, Sigma-Aldrich, St. Louis, MO) was dissolved in 50% aqueous ethanol (treatment IBA/EtOH).

Liquid auxin treatments were applied by dipping the basal 1 cm (0.4 in) of cuttings in the treatment solution for 5 s. Treated cuttings were allowed to air dry for 5 min to allow the auxin to adhere to the stem tissue. Powder formulations were applied by dipping basal 1 cm (0.4 in) of cutting in powder and then gently tapping cutting to remove excess talc. For all experiments a cutting was considered rooted if it had at least one root greater than 0.2 cm (0.08 in) in length (to distinguish roots from callus). For further information regarding auxin products registered for plant propagation and their use, see Boyer et al. (2013).

Experiments 1 to 3. To determine the influence of stock plant growth stage, softwood (June 16, 2011) (expt. 1), semi-hardwood (August 5, 2011) (expt. 2), and hardwood (February 20, 2012) (expt. 3) cuttings were treated with K-IBA at 0; 2,500; 5,000; or 10,000 ppm (0, 0.25, 0.5, or 1.0%) in water. The experimental design was a randomized complete block design with 5 cuttings (subsamples) per K-IBA treatment and 8 replications. Cuttings were harvested and data collected after 18 weeks (softwood), 11 weeks (semi-hardwood), and 12 weeks (hardwood). Data included percent rooting, root number per rooted cutting, and total root length per rooted cutting.

Experiments 4 and 5. To determine the influence of liquid or talc-powder auxin application method, softwood (June 7, 2012) (expt. 4) and semi-hardwood (July 27, 2012) (expt. 5) cuttings were treated with 0, 2,500, 5,000, or 10,000 ppm (0, 0.25, 0.5, or 1.0%) K-IBA/H₂O or 1,000, 3,000, or 8,000 ppm (0.1, 0.3, or 0.8%) IBA/talc. The experimental design was a randomized complete block design with 5 cuttings (subsamples) per treatment and 7 replications. Cuttings were harvested and data collected after 20 weeks (softwood) and 16 weeks (semi-hardwood). Data included percent rooting, root number of rooted cuttings and total root length of rooted cuttings.

Experiment 6. To investigate the influence of water and ethanol as solvents, semi-hardwood cuttings (June 26, 2013) were treated with 0, 2,500, 5,000, 10,000, or 15,000 ppm (0, 0.25, 0.5, 1.0, or 1.5%) K-IBA/H₂O or IBA/EtOH. The experimental design was a randomized complete block design with a factorial arrangement of treatments (5 auxin concentrations times 2 solvents). Each treatment contained 5 cuttings (subsamples) and was replicated 8 times except 15,000 ppm (1.5%) IBA/EtOH, which had only 5 replications due to a shortage of cuttings. Cuttings received 4 preventative fungicide drench treatments at 1-week intervals from June 27 to August 1 using a rotation of mefenoxam [active ingredient at $2.6 \text{ mL} \cdot \text{L}^{-1}$ ($\overline{0.33} \text{ fl oz} \cdot \text{gal}^{-1}$) (Mefenoxam 2AQ, Quali-Pro, Pasadena, TX)], thiophanate methyl [active ingredient at 304 mg·L⁻¹ (0.04 oz·gal⁻¹) (3336WP, Cleary Chemicals LLC, Dayton, NJ)], and azoxystrobin [active ingredient at 22.5 mg·L⁻¹ (0.003 oz·gal⁻¹) (Heritage Fungicide, Syngenta Group Company, Greensboro, NC)]. After 16 weeks, cuttings were harvested and percent rooting, root number of rooted cuttings, and total root length of rooted cuttings were recorded.

Experiment 7. To determine the effect of cutting position on rooting, experiment 6 was repeated with second-flush softwood (August 14, 2013) shoot tip cuttings and subterminal cuttings. The experimental design was a randomized complete block design with a three-way factorial arrangement of treatments (5 auxin concentrations times 2 solvents times 2 cutting positions). There were 5 cuttings (subsamples) per treatment and 8 replications. In this experiment each replication represented a different stock plant (genotype) except for the subterminal cuttings where genotypes were combined to create 4 replications due to a lack of stock plant material. Preventative fungicide treatments were applied as mentioned above. After 11 weeks, cuttings were harvested and percent rooting, root number of rooted cuttings, and total root length of rooted cuttings were recorded.

Statistical analysis. Data were subjected to analysis of variance using PROC GLM procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC). Where appropriate, data were also subjected to regression analysis.

Results and Discussion

Experiments 1 to 3. K-IBA/H₂O treatment did not affect percent rooting, root number, or root length of stem cuttings at any growth stage (data not shown). Rooting averaged 8.8% (P = 0.45) for softwood cuttings and 44.4% (P = 0.61) for semi-hardwood cuttings. Hardwood cuttings did not root (0%). Average root number per rooted cutting was 1.5 (P = 0.22) and 2.4 (P = 0.29) for softwood and semi-hardwood cuttings, respectively. Root length averaged 13.0 cm (5.1 in) (P = 0.70) for softwood cuttings.

Experiments 4 and 5. Auxin formulation (liquid or powder) did not influence rooting percent, root number, or average root length of softwood or semi-hardwood stem cuttings (data not shown). Softwood cuttings did not root (0%). Rooting of semi-hardwood cuttings averaged 14.3% (P = 0.36) and 12.4% (P = 0.34), root number averaged 1.4 (P = 0.61) and 1.8 (P = 0.43), and root length averaged 11.1 cm (4.4 in) (P = 0.75) and 10.3 cm (4.1 in) (P = 0.61) for the K-IBA/H₂O and IBA/talc treatments, respectively.

Experiment 6. Auxin concentration clearly influenced rooting of semi-hardwood cuttings in 2013 (P = 0.0002) (Table 1). Rooting percentage decreased linearly as auxin

Table 1. Percent rooting of semi-hardwood terminal stem cuttings of Acer truncatum treated with either the potassium (K) salt of indole-3-butyric acid (K-IBA) dissolved in water (H₂O) or the free acid of IBA dissolved in ethanol (EtOH):water (1:1 v/v)².

Auxin (ppm)	2011 K-IBA/ H ₂ O	2012 K-IBA/ H ₂ O	2013	
			K-IBA/ H ₂ O	IBA/ EtOH
0	42.5	22.9	37.1	48.6
2,500	30.0	22.9	25.7	25.7
5,000	55.0	8.6	20.0	28.6
10,000	50.0	2.9	14.3	14.3
15,000	_	—	2.9	10.0
ANOVA	NS ^y	NS	**	*
Linear regression		_	**	*

²Cuttings were 15 cm (6 in) long, inserted into perlite:peat moss (3:1 v/v) substrate, and rooted on a greenhouse bench under intermittent mist. ³Nonsignificant (NS) at $P \le 0.05$, (*) Significant at $P \le 0.05$, or (**) Significant at $P \le 0.01$;

n = 40, 35, and 35 cuttings per treatment for 2011, 2012, and 2013, respectively.

concentration increased regardless of solvent type (H₂O or EtOH). Mean root number (1.6 for K-IBA/H₂O and 2.3 for IBA/EtOH) (P = 0.15) and mean root length [6.6 cm (2.6 in) for K-IBA/H₂O and 7.9 cm (3.1 in) for IBA/EtOH] (P = 0.65) were unaffected by either auxin concentration or solvent (data not shown). There was no significant interaction between auxin concentration and solvent.

Experiment 7. Auxin concentration, solvent, and cutting position did not significantly influence rooting percentage (P = 0.21), root number (P = 0.06), or average root length (P = 0.27) of second-flush softwood cuttings (data not shown). Rooting of K-IBA/H₂O treatments averaged 5 and 1.6% for terminal and subterminal cuttings, respectively. Rooting of IBA/EtOH treatments averaged 10 and 7.2% for terminal and subterminal cuttings, respectively. Overall, shoot tip cuttings rooted at 7.5% whereas subterminal cuttings rooted at 4.8%. No interaction was observed between auxin concentration, solvent, or cutting position. Overall root number per rooted cutting was 2.0 with an average root length of 6.6 cm (2.6 in).

Timing. Results from experiments 1 through 5 suggest that maximum rooting potential for terminal stem cuttings occurs during the semi-hardwood stage of shoot development. The results showed considerable variability from year to year. For example, semi-hardwood cuttings rooted at 44% in 2011, but only 14% in 2012, suggesting proper wood maturity for optimum adventitious root promotion is specific and of limited time. Similar to the current study, Pair (1986) found that semi-hardwood stem cuttings of shantung maple rooted better than softwood cuttings. Vertrees (1978) attributed failed rooting of shantung maple to poor timing of cutting collection. He noted that tender cuttings and excessively mature cuttings tend to root poorly. Working in Michigan, Chapman (1979) found that percent rooting of stem cuttings from hedge maple (A. campestre L.) and Norway maple (A. platanoides L.) briefly peaked (75 and 85%, respectively) in mid-June. In that study, cuttings of both species rooted poorly ($\leq 40\%$) before and after peak dates. This emphasizes the importance of propagating maples during their ideal developmental stage. Results from the current study suggest that optimal rooting of shantung maple occurs near the time when shoots have finished elongating, leaves near the apex have fully expanded, and stem tissue has lignified.

Auxin concentration. Of the seven sets of cuttings observed in this research, only the semi-hardwood cuttings of 2013 showed a significant response to auxin concentration (Table 1). In that case, percent rooting decreased as auxin concentration increased. This shows that rooting was not hindered by a lack of auxin. The lack of similar results from the second-flush softwood cuttings of 2013 (data not shown) was likely due to collecting cuttings when they were too tender.

The condition of stock plants in this study best explains the varied responses of semi-hardwood stem cuttings to auxin concentration (Table 1). In 2011, when semi-hardwood cuttings showed no response to auxin concentrations, stock plants had recently been topped at 1.5 m (4.9 ft). In 2012, differences in semi-hardwood rooting were nonsignificant, but rooting percentages appeared to favor low auxin concentration or no auxin. In 2013, high auxin concentrations significantly reduced rooting. Thus, during the three seasons of this study, semi-hardwood stem cuttings increased in sensitivity to auxin concentration. During this time, stock plants were annually pruned significantly to supply cutting material, which may have rejuvenated stock plants. Support for this hypothesis is provided in work by Podaras and Bassuk (1996) where a 4-year-old greenhouse-grown shantung maple showed greater sensitivity to auxin concentration than a 16-year-old field-grown specimen.

Further research is needed to clearly determine the influence of ontogenic aging on the response of shantung maple stem cuttings to auxin concentration. The study by Podaras and Bassuk (1996) also showed that low concentrations of auxin between 0 and 1,000 ppm (0 to 0.1%) may encourage rooting of shantung maple cuttings. The lowest auxin concentration applied in the current study was 2,500 ppm (0.25%). In most instances there was no statistical difference in rooting between 0 and 2,500 ppm (0 to 0.25%) IBA or K-IBA, even though a greater number of cuttings rooted with no auxin application. A study with auxin concentrations ranging from 0 to 2,500 ppm (0 to 0.25%) may produce better rooting results.

Interestingly, root number and average root length of semi-hardwood cuttings of 2013 show no response to auxin concentration. Davis and Haissig (1990) note that roots arise from either preformed or induced root primordia. Fink (1982) states that species of maple (*Acer L.*) possess preformed root primordia. In the current study, auxin neither hindered preformed primordia development nor induced additional primordia development as demonstrated by the consistent root number. Uniform root length indicates treatments rooted simultaneously. Similar to work by Chong (1981), high auxin concentrations did not hinder root elongation.

Formulation. Similar rooting of cuttings treated with liquid or powder auxin formulations was unexpected. Normally, liquid formulations provide better rooting than powder formulations (Hartmann et al. 2011). Cuttings likely absorb less auxin from powder formulations than from liquid formulations, leading to differences in rooting success. Because auxin concentration did not affect rooting in semi-hardwood stem cuttings of 2012, formulation differences did not affect rooting either.

Solvent. Solutions containing ethanol can improve auxin uptake by stem cuttings (Heung and McGuire 1973), but may damage tender tissues (Hartmann et al. 2011). In this study, differences in rooting, root number, and root length were not related to solvent. Further work could investigate an increase in the concentration of ethanol or include other solvents such as dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG), which improved root number and length in work by Dirr (1989).

Cutting position. Subterminal cuttings were included in this study because results from Pair (1986) suggested that subterminal cuttings root slightly better (69%) than terminal cuttings (55%). Haissig (1972) found that brittle willow (*Salix fragilis* L.) develops root primordia at subterminal nodes as shoots elongate. This should cause subterminal cuttings to root better than terminal cuttings. In the current study, terminal and subterminal cuttings showed no differences in rooting. Either subterminal cuttings of shantung maple have

no rooting advantage over terminal cuttings, or the maximum rooting potential of subterminal cuttings was reduced in this study by their tender condition, which were only investigated as second-flush softwood cuttings. Nevertheless, subterminal cuttings root at least as well as terminal cuttings. Using both terminal and subterminal cutting material for propagation could speed the establishment of clonal populations of future shantung maple selections.

This report describes a method where mature shantung maple can be encouraged to produce numerous vigorous shoots that are appropriate to use as stem cuttings. Hedging may be more successful with juvenile seedling stock plants; however, when cloning desirable ornamental characteristics of a mature established plant is the goal, hedging was successful.

Rooting efficiency from mature shantung maples remains low. Successful propagation depends primarily on collecting stem cuttings at their optimum developmental stage, which occurs near the time when shoots lignify and leaves at the shoot apex reach their mature size. This study suggests that treating cuttings with auxin concentrations > 2500 ppm (0.25%) will not improve rooting and may decrease rooting. Applying no auxin often resulted in the highest percent rooting in the current study.

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