Vegetative Propagation of Mature Eastern and Carolina Hemlocks by Rooted Softwood Cuttings

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Abstract. This study tested the effects of cutting length and auxin (NAA) concentration on adventitious root formation in softwood stem cuttings from mature eastern hemlock, *Tsuga canadensis* (L.) Carr., and carolina hemlock, *T. caroliniana* Engelm. Overall rooting percentage (41%) and percent mortality (22%) were higher for eastern hemlock compared with carolina hemlock (10% rooting and 13% mortality). Rooting percentage of each species responded differently to varying auxin concentrations (0, 1, 2, 4, 8 mM NAA). Maximum rooting (56%) for eastern hemlock occurred at 0 mM NAA; then decreased with increasing auxin concentration. Carolina hemlock rooting percentage increased from the control to a maximum (16%) at 1 mM NAA; then decreased with increasing auxin concentration. For both species, the lowest mortality occurred at the same auxin concentration as maximum rooting. The highest rates of mortality coincided with the same concentrations as the lowest rooting percentages. At all auxin concentrations, eastern hemlock had a higher number of roots and greater total root length relative to carolina hemlock. Mortality among 6-cm stem cuttings was twice that observed for 3-cm cuttings of both species. However, 6-cm cuttings of eastern hemlock that did form adventitious roots had more roots and longer total root length compared with 3-cm cuttings. Chemical name used: 1-naphthalenacetic acid.

Recent interest in the vegetative propagation of hemlock (*Tsuga* spp.) by rooted cuttings arises primarily from the threat posed by the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), on two species: eastern hemlock, *Tsuga canadensis* (L.) Carr., and carolina hemlock, *T. caroliniana* Engelm. The adelgid is an exotic pest with the potential to cause the extinction of both species. First reported and described by Annand (1924) in the Pacific Northwest from specimens collected on western hemlock, *T. heterophylla* (Raf.) Sarg., this pest is believed to have originated in Asia (McClure 1987; Takahashi 1937). Introduced into the eastern United States (Virginia) on imported nursery stock in the early 1950s, HWA had spread into natural stands by the 1980s. The adelgid has since caused widespread mortality of both eastern and carolina hemlock throughout 13 eastern states, killing trees in as little as 4 years (McClure et al., 2001).

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The development of techniques for the vegetative propagation of eastern and carolina hemlock, as well as other *Tsuga* spp., will be of great utility to current and future effort in HWA control and research. Current biological control programs depend on field collection of HWA infested hemlock branches, often from remote sites, for the mass rearing of adelgid predators and this has been recognized as a major limitation to production (Cheah et al., 2004; Palmer and Sheppard, 2002). The ability to root cuttings from these species will allow for relatively small field collections that can be vegetatively multiplied and infested with HWA to provide an ample, easily assessable, and local source of host material for predator rearing. Given the low seed viability for both species (<25%), vegetative propagation via rooted cuttings could also be used in addition to seed collection for current gene conservation efforts for eastern and carolina hemlock (Godman and Lancaster, 1991; Romero et al., 2000; Staniforth, 2001). This technology will also benefit those who propagate, breed, and sell the 80-plus eastern and carolina hemlock ornamental cultivars (Ouden and Boom 1982; Swartley 1984).

Future efforts to understand hemlock host resistance against HWA will benefit from this technology as well. Although HWA can kill hemlocks in as little as 4 years, there are some trees that have survived infestation for nearly 20 years. At this time it is unclear if this is a result of site conditions or moderate levels of host resistance (McClure et al., 2001; Souto et al., 1996). The ability to root stem cuttings from these surviving hemlocks will be an important tool for determining the basis of this prolonged survival, providing an

increased number of trees and genotypes for study that will grow and mature more rapidly than propagules from seed. When hemlock host resistance is understood, the availability of rooted cutting technology will accelerate the breeding and deployment of resistant trees to areas devastated by HWA. While grafting would offer similar opportunities and benefits for the vegetative propagation of hemlocks, rooted cuttings are a more cost effective and less time consuming technique.

Currently, there is a body of information concerning the propagation of semi-dormant and dormant hardwood stem cuttings from eastern, western, and carolina hemlocks (Doran, 1952; Flint and Jesinger, 1971; Fordham, 1971; Mitsch, 1975; Packee, 1991; Swartley, 1984; del Tredici, 1985; Waxman, 1985; Wigmore and Woods, 2000). However, there is relatively little known about the cultural techniques, specifically auxin concentrations, necessary for the successful rooting of softwood cuttings from these species. While indole-3-butyric acid (IBA) or IBA plus 1-naphthalenacetic acid (NAA) are more commonly used to root conifer stem cuttings, NAA alone is effective in promoting adventitious root formation in a number of species, including eastern hemlock, loblolly pine (*Pinus taeda* L.), eastern white pine (*Pinus strobus* L.), and Fraser fir (*Abies fraseri* (Pursh) Poir) (Diaz-Sala et al., 1996; Doran, 1952; Goldfarb et al., 1998a, 1998b; Rosier, 2003). Therefore, the objective of this study was to test the effects of cutting length and NAA concentration on adventitious root formation in softwood stem cuttings of eastern and carolina hemlock.

Materials and Methods

Plant material. Eastern hemlock stem cuttings were collected from trees growing along 15 miles (mile posts 261 to 275) of the Blue Ridge Parkway in Ashe County, N.C. Carolina hemlock stem cuttings were collected from trees growing beside the South Toe River at the Carolina Hemlocks Recreation Area (Pisgah National Forest) in Yancey County, N.C. Softwood cuttings were harvested from primary branch tips in the lower crown on 24 and 25 June 2002. Six cuttings were taken from each of about 120 trees per species. Following collection, each cutting was immediately wrapped in a moistened paper towel and placed on ice. Cuttings were transported to Raleigh, N.C., on 26 June 2002 and stored at 4 °C until used in the rooting trial.

Rooting trial treatments and experimental design. The rooting trial tested the effects of the two cutting lengths (3 and 6 cm) and five concentrations of NAA (0, 1, 2, 4, 8 mM). Beginning with a stock solution of 8 mM NAA, each auxin concentration was prepared by serial dilution into a 50% isopropyl alcohol-deionized water solution. The control (0 mM NAA) consisted of the 50% isopropyl alcohol-deionized water solution. The prepared auxin solutions were placed into opaque bottles and stored at 4 °C until used the same day. On 28 June 2002, the stem cuttings were recut from the base to either 3 or 6 cm, and auxin was applied by a 3

Table 1. Analysis of variance for traits assessed in a rooting trial of eastern and carolina hemlock softwood stem cuttings collected from mature trees. *F* values with indicated level of significance are given for each trait.

Source of variation	df ²	Rooting (%)	Mortality (%)	Primary roots (no.)	Total length of primary roots (cm)
Block	7	0.99 ^{NS}	2.26*	2.45*	1.54 ^{NS}
Species	1	161.01***	9.33**	16.59***	20.90***
AC ³	4	12.33***	8.36***	6.07***	2.05 ^{NS}
Species × AC	4	5.73***	0.48 ^{NS}	1.68 ^{NS}	1.06 ^{NS}
CL ⁴	1	1.14 ^{NS}	21.11***	21.53***	32.28***
Species × CL	1	2.21 ^{NS}	0.83 ^{NS}	4.81*	23.50***
AC × CL	4	0.6 ^{NS}	1.81 ^{NS}	2.09 ^{NS}	1.35 ^{NS}
Species × AC × CL	4	1.17 ^{NS}	0.96 ^{NS}	0.03 ^{NS}	1.34 ^{NS}

²Degrees of freedom.

³Auxin concentration.

⁴Cutting length.

^{NS},*,***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Analysis of variance for traits assessed individually for species in a rooting trial of eastern and carolina hemlock softwood stem cuttings collected from mature trees. *F* values with indicated level of significance are given for each trait.

Source of variation	df ²	Rooting (%)	Mortality (%)	Primary roots (no.)	Total length of primary roots (cm)
Eastern hemlock					
Block	7	0.81 ^{NS}	1.49 ^{NS}	2.08 ^{NS}	1.21 ^{NS}
AC ³	4	10.40***	3.58*	3.28*	4.09**
CL ⁴	1	2.06 ^{NS}	13.09***	41.91***	95.24***
AC × CL	4	0.60 ^{NS}	0.94 ^{NS}	1.78 ^{NS}	3.35*
Carolina hemlock					
Block	7	1.19 ^{NS}	3.07**	1.16 ^{NS}	0.50 ^{NS}
AC	4	3.66**	6.22***	6.12**	0.60 ^{NS}
CL	1	0.20 ^{NS}	8.98**	1.66 ^{NS}	0.63 ^{NS}
AC × CL	4	1.88 ^{NS}	2.23 ^{NS}	0.77 ^{NS}	0.11 ^{NS}

²Degrees of freedom.

³Auxin concentration.

⁴Cutting length.

^{NS},*,***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

s dip of the basal 1 cm. Cuttings were allowed to air dry for 15 min before being inserted to a depth of 2 cm into a rooting medium consisting of 3 horticultural perlite : 2 peat (v/v). Cuttings were rooted individually in 164-mL Ray Leach SC-10 Super Cells (Stuewe & Sons, Inc., Corvallis, Ore.).

The experiment was a randomized complete block design with eight blocks, each containing 20 treatment combinations with eight cuttings per treatment for a total 1280 cuttings. The entire study was surrounded by a row of border cuttings. The rooting trial lasted 24 weeks (28 June 2002 to 28 Dec. 2002) after which rooting percentage and percent mortality among all cuttings as well as the number of primary roots and total length of primary roots (cm) among rooted cuttings were recorded. The experiment was not repeated in time.

Rooting environment. The rooting trial was conducted in a clear polyethylene-covered greenhouse located at the N.C. State University Horticultural Field Laboratory, Raleigh. Cuttings were rooted under natural photoperiod and irradiance, except for the summer period (28 June 2002 through August 2002) when irradiance was reduced 60% by placing shade cloth on the greenhouse. Heating and cooling systems maintained the daily air temperature between 23 and 26 °C and the night temperature between 20 and 23 °C for the duration of the rooting trial. Cuttings were maintained under intermittent mist at a frequency varied inversely

to the relative humidity within the greenhouse using a Grower Junior (McConkey Co., Mt. Puyallup, Wash.) overhead boom irrigation system. Mist delivery rate was held constant at 61 mL·m⁻² from 28 June 2002 through 31 Aug. 2002 and 53 mL·m⁻² from 1 Sept. 2002 through 28 Dec. 2002.

Statistical analysis. Analysis of variance was performed using the General Linear Model procedure (PROC GLM) of SAS version 8.1 (SAS Institute, 1999). The main effects of block, species, auxin concentration, cutting length, and all two- and three-way interactions other than those including block were tested for all rooting traits assessed (Table 1). Also tested were the main effects of block, auxin concentration, cutting length, and the interaction between auxin concentration and cutting length on rooting traits assessed for each species individually (Table 2). The variables rooting percentage and percent mortality were transformed using the arcsine of the square root to increase homogeneity among treatment variances. Differences between the transformed and nontransformed data were negligible; therefore, all results presented are based on nontransformed data. All means are reported as least squares means.

Results and Discussion

Effect of hemlock species on adventitious root formation. Rooting percentage, percent

mortality, number of primary roots, and total length of primary roots were all significantly affected by the main effect of species (Table 1). Overall, eastern hemlock softwood stem cuttings rooted at a higher rate (41%) than those of carolina hemlock (10%). While this result might indicate some inherent difference in rooting capacity between these two species, our study was not designed to investigate this possibility. Additional explanations for differential rooting between eastern and carolina hemlock include variation in stock plant age and site type. Both species occur on nutrient poor soils, but eastern hemlock sites are typified by very moist, well-drained soils and carolina hemlock sites by very dry, rocky soils (Godman and Lancaster 1990, Rentch et al. 2000). This difference in soil moisture availability could have affected the water status of stem cuttings at the time of collection and may at least partially account for the observed differences in stem cutting rooting rates.

Overall mortality among softwood stem cuttings was 22% for eastern hemlock and 13% for carolina hemlock. In total, 37% of eastern hemlock stem cuttings and 77% of carolina hemlock stem cuttings that did not root were alive at the end of the 6-month trial. This indicates that carolina hemlock softwood stem cuttings might require more time or altered cultural conditions to root at rates equivalent to eastern hemlock.

The number of primary roots and total primary root length among eastern hemlock stem cuttings were 2.6 roots per cutting and 8.6 cm, respectively, while these variables were nonestimable for carolina hemlock. The nonestimable means in the analysis were a result of the extremely low overall rooting percentage among carolina hemlock stem cuttings, particularly in the 8 mM NAA treatments (3%).

Effect of cutting length on adventitious root formation. Percent mortality, number of primary roots, and total length of primary roots were all significantly affected by the main effect of cutting length (Table 1). Significant interactions between species and cutting length were detected for number of primary roots and total length of primary roots (Table 1). There was no effect of cutting length on rooting percentage for either species (Tables 1 and 2, Fig. 1a). Mortality among larger cuttings of both eastern and carolina hemlock was twice that observed among smaller cuttings, with overall mortality of 24% among 6-cm cuttings compared with 11% among 3-cm cuttings (Fig. 1b). Increased mortality among 6-cm cuttings may be attributable to a greater exposed length (4 cm) above the soil compared with 3-cm cuttings (1 cm). Cuttings with greater exposed length have more above-soil leaf area that may be subjected to higher rates of transpirational stress. Such stress may have caused mortality directly through desiccation or indirectly through the inhibition of adventitious root formation.

Significant interactions between species and cutting length for the mean number of primary roots and mean length of primary roots were detected. Due to the extremely low rooting percentage for 3-cm carolina hemlock stem cuttings the means were nonestimable (Fig. 1c

Fig. 1. Effect cutting length on (A) rooting percentage, (B) percent mortality, (C) number of primary roots, and (D) total length of primary roots of eastern and carolina hemlock softwood stem cuttings. The number of primary roots and total root length of 3 cm carolina hemlock cuttings were nonestimable. Cutting length: 3 cm (■), 6 cm (□).

and d). Therefore, the following discussion of cutting length and its effects on root number and length will focus on results for eastern hemlock only.

The number of primary roots and total length of primary roots among eastern hemlock softwood stem cuttings were significantly affected by cutting length (Table 2), with fewer (2.0) and shorter (5.1 cm) roots formed on 3-cm cuttings compared with 6-cm cuttings (3.5 and 12.3 cm) (Fig. 1c and d). Similar responses have been reported for stem cuttings of both sweetgum, *Liquidambar styraciflua* L., and fraser fir where longer cuttings or cuttings of greater diameter produced more roots than shorter or thinner cuttings (Miller et al., 1982; Rieckermann et al., 1999). While greater above ground leaf surface area may lead to greater transpirational stress and subsequent mortality, it may also benefit cuttings that successfully root providing a larger pool of photosynthetic resources for root formation. Cuttings of larger size and greater length may also have greater levels of stored carbohydrates for root growth (Rieckermann et al., 1999).

Effect of auxin concentration on adventi-

tious root formation. Rooting percentage, percent mortality, and number of primary roots were all significantly affected by the main effect of auxin concentration with a significant interaction between species and auxin concentration detected for rooting percentage (Table 1). The application of exogenous auxin is often an effective and, sometimes, necessary treatment for promoting adventitious root formation in conifer stem cuttings (Hinesley and Blazich, 1981). This beneficial effect of auxin treatment was detected for softwood stem cuttings of carolina hemlock. Rooting was significantly affected by auxin concentration (Table 2) increasing from 8% for the control to a maximum of 16% at 1 mM NAA then subsequently decreasing with increasing auxin concentration to 4% at 8 mM NAA (Fig. 2a). It appears, however, that exogenous auxin treatments lack any benefit for rooting in eastern hemlock softwood stem cuttings. Rooting was significantly affected by auxin concentration (Table 2) with a maximum of 56% occurring at 0 mM NAA followed by a decrease with increasing auxin concentration to a low of 19% at 8 mM NAA (Fig. 2a).

Overall, mortality decreased from 13% at 0 mM NAA to 11% at 2 mM NAA and then increased with increasing auxin concentration to a high of 32% at 8 mM NAA. The number of roots per cutting increased with increasing auxin concentration from 1.5 at 0 mM NAA to 2.5 at 4 mM NAA. The number of primary roots at 8 mM NAA was nonestimable for both the overall analysis and carolina hemlock. The mortality and root number responses of the individual species to auxin concentration are shown in Fig. 2b and c.

The total primary root length for eastern hemlock softwood stem cuttings was significantly affected by auxin concentration (Table 2). The longest average root length of 10.8 cm occurred at 2 mM NAA decreasing with decreasing and increasing auxin concentration to 7.2 cm and 7.0 cm at 0 mM NAA and 8 mM NAA, respectively (Fig. 2d). The effect of NAA concentration on root length of carolina hemlock softwood stem cuttings (Fig. 2d) was not significant ($P = 0.66$, Table 2). The number of primary roots at 8 mM NAA was nonestimable among carolina hemlock owing to the overall low rooting percentage.

Fig. 2. Effect of NAA concentration on (A) rooting percentage, (B) percent mortality, (C) number of primary roots, and (D) total length of primary roots of eastern (—) and carolina (----) hemlock softwood stem cuttings. The number of primary roots and total root length of carolina hemlock cuttings at 8 mM NAA were nonestimable.

Conclusion

To date, there has been no direct experimental test of the effect of cutting type (softwood versus hardwood) on rooting in eastern and carolina hemlock stem cuttings. Overall, softwood stem cuttings of eastern and carolina hemlock rooted at rates much lower than those reported for dormant and semi-dormant hardwood cuttings from these species. Doran (1952) reported mean rooting rates of 65% and 71% for hardwood stem cuttings, treated with comparable concentrations of auxin (0.5 to 1.0 mM IBA alone or NAA alone), of eastern and carolina hemlock, respectively. However, direct comparisons between the current study and Doran's are tenuous.

The results of this trial are encouraging and indicate that softwood stem cuttings from eastern hemlock can be rooted at reasonably high rates without exogenous auxin treatments. Additionally, it is clear that cutting size is an important mediating factor for successful rooting in this species, with larger cuttings producing more and larger roots. Rooting rates for carolina hemlock were significantly lower than those of eastern hemlock, but maximum rooting occurred at 1 mM NAA, indicating that

very little auxin is required to root softwood cuttings from this species. The high survival rate among carolina hemlock cuttings that did not root suggests that additional time in the rooting environment or slightly altered cultural conditions may improve rooting rates.

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