Propagation of 'Carolina Sapphire' Smooth Arizona Cypress by Stem Cuttings: Effects of Growth Stage, Type of Cutting, and IBA Treatment[1]

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Abstract

Stem cuttings of 'Carolina Sapphire' smooth Arizona cypress [*Cupressus arizonica* var. *glabra* (Sudw.) Little 'Carolina Sapphire'] consisting of 30 cm (12 in) terminals or distal [terminal 15 cm (6 in)] and proximal [basal 15 cm (6 in)] halves of 30 cm (12 in) terminals were taken on three dates associated with specific growth stages (semi-hardwood, hardwood, and softwood). Cuttings were treated with indolebutyric acid (IBA) in 50% isopropanol ranging from 0 to 16,000 ppm (1.6%) and placed under intermittent mist. Regardless of cutting type and auxin treatment, cuttings rooted at each growth stage. Overall percent rooting was highest during the hardwood stage (70%), followed by the semi-hardwood stage (44%). Softwood cuttings exhibited the lowest overall rooting (33%). At each growth stage, percent rooting, root count, and root dry weight varied depending on cutting type and IBA treatment. Rooting percentages ≥70% were attained at the semi-hardwood and hardwood growth stages for particular treatment combinations.

Index words: auxin, indolebutyric acid, adventitious rooting, *Cupressus arizonica* var. *glabra*.

Significance to the Nursery Industry

'Carolina Sapphire' smooth Arizona cypress is a fast growing, attractive evergreen tree with considerable potential for use in the landscape and as a Christmas tree. Although interest and subsequent demand for this cultivar are increasing, supplies are limited due in part to propagation difficulties.

Results herein indicate that stem cuttings of 'Carolina Sapphire' smooth Arizona cypress can be rooted at any growth stage with semi-hardwood and hardwood cuttings capable of rooting at percentages ≥70%. However, at each growth stage rooting percentage, root count, and root dry weight varied, depending on cutting type and IBA treatment.

Introduction

'Carolina Sapphire' smooth Arizona cypress (*Cupressus arizonica* var. *glabra* 'Carolina Sapphire') is a rapid growing tree with young plants producing up to 2 m (6 ft) of growth yearly (7). It displays a broadly pyramidal crown, a loose growth habit, and steel blue foliage. In recent years this cultivar has attracted much attention in the southeast United States because of possible use as a fast growing screen with interesting color attributes for the landscape. In addition, 'Carolina Sapphire' has considerable merit as a Christmas tree, and the Christmas tree industry is beginning to cultivate this particular cultivar in plantations.

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Propagation of 'Carolina Sapphire' is occurring presently at nurseries scattered throughout the southeast United States. However, the level of success varies from one nursery to another (L.E. Hinesley, personal communication). Difficulties regarding propagation are related in part to lack of definitive research. Little research has been reported on vegetative propagation by stem cuttings. What research has been published on 'Carolina Sapphire' and closely related species is sketchy, and simply indicates that stem cuttings can be rooted (2, 6, 9). If the landscape and Christmas tree potentials of 'Carolina Sapphire' are to be realized, research on vegetative propagation is needed. Therefore, the following study was conducted to develop a protocol for propagation of 'Carolina Sapphire' smooth Arizona cypress by stem cuttings. Specifically, the influence of timing (growth stage), type of cutting, and indolebutyric acid (IBA) treatment on rooting were investigated.

Methods and Materials

Terminal cuttings approximately 35 cm (15 in) in length were taken from six, 5-year-old trees growing in the North Carolina State University Arboretum, Raleigh, on three dates associated with specific growth stages: November 10, 1994 (semi-hardwood), February 24, 1995 (hardwood), and May 12, 1995 (softwood). Shoot growth began approximately the first week of April. Sixty cuttings per tree were taken on November 10, 1994, and February 24, 1995, and 72 cuttings per tree were taken on May 12, 1995. Cuttings were taken throughout the crown of each tree. The trees were growing in soil of uniform fertility and had not been sheared.

As cuttings were collected, they were placed in plastic bags; the bags were placed on ice and transported to the Horticultural Science Greenhouses, Raleigh, NC. After collection, all cuttings were pooled, randomized, and trimmed resulting in the following groups: a) entire 30 cm (12 in) terminal cuttings, b) distal halves [terminal 15 cm (6 in)] of 30 cm (12 in) terminal cuttings or c) proximal halves [basal 15 cm (6 in)] of 30 cm (12 in) terminal cuttings. Before auxin treatment, branches were pruned with hand shears from the basal 6 cm (2.4 in) of the 30 cm (12 in) intact terminals and for the 15 cm (6 in) distal and 15 cm (6 in) proximal halves, branches were removed similarly from the basal 4 cm (1.6 in). Foliage from the lower portion of the cuttings was removed by hand. The basal 1 cm (0.4 in) of the semi-hardwood and hardwood cuttings were then treated for 1 to 2 sec with 0, 4,000 (0.4%), 8,000 (0.8%), 12,000 (1.2%), or 16,000 ppm (1.6%) IBA (reagent grade IBA in 50% isopropanol). Softwood cuttings received the same treatments with an additional treatment of 2,000 ppm (0.2%) IBA. The cuttings were air dried for 15 min before insertion into a raised greenhouse bench containing a nonheated medium of peat:perlite (1:1 by vol). Intact terminal cuttings were inserted to a depth of 6 cm while proximal and distal cuttings were inserted to 4 cm (1.6 in). The experimental design within the propagation bed for semi-hardwood and hardwood cuttings was a randomized complete block with a factorial arrangement of treatments (three cutting types × five IBA levels), six blocks, and six cuttings per treatment per block. The design for the softwood cuttings was identical except there were six levels of IBA.

Cuttings were maintained under natural photoperiod and irradiance with days/nights of 27 ± 5C (80 ± 9F)/21 ± 5C (70 ± 9F). Intermittent mist operated daily for 6 to 8 sec every 5 min from sunrise to sunset. The mist was delivered by deflection type nozzles with a capacity of 32.2 liters (8.5 gal) water/hr. To control fungi, cuttings were sprayed initially and weekly thereafter alternating ethylene biodithiocarbamate ion (FORE) and chlorothalonil (Daconil) at 2 ml/liter (0.52 tbs/gal) and 2.4 g/liter (1.0 tbs/gal), respectively.

Cuttings were harvested after 18 weeks for each growth stage. For the semi-hardwood and hardwood cuttings, data were recorded on percent rooting, number of primary roots ≥1 mm (0.04 in), total root length, total root area, and root dry weight [dried at 70C (158F) for 72 hr]. Similar data were recorded for the softwood cuttings except for total root length and total root area. All data except rooting percentage were based on the actual cuttings that rooted (at least one primary root). Before drying the roots of semi-hardwood and hardwood cuttings, total root length and root area were measured using a Monochrome Agvision System 286 Image Analyzer (Decagon Devices, Inc., Pullman, WA). Data were subjected to analysis of variance and regression analysis, and a linear contrast was used to test for differences between pooled IBA treatments (4,000, 8,000, 12,000, and 16,000 ppm IBA) and nontreated cuttings (0 ppm IBA) (8).

Results and Discussion

Stem cuttings of 'Carolina Sapphire' smooth Arizona cypress rooted at each growth stage (Table 1). Depending on the growth stage, the influence of cutting type and IBA treatment on various rooting measurements were variable (Tables 2–6). At the semi-hardwood and softwood growth stages there were no interactions between IBA treatment and cutting type for all measurements of rooting. However, at the hardwood growth stage, there was a highly significant interaction ($P \leq 0.01$) between IBA treatment and cutting type for root count.

Overall, percent rooting was highest for the hardwood cuttings followed by the semi-hardwood cuttings (Table 1). Softwood cuttings exhibited the poorest rooting. The influence of cutting type on rooting was similar for hardwood and semi-hardwood cuttings, with distal halves providing the greatest rooting, followed by the proximal halves and the entire terminals (Table 1). Cutting type had no significant influence on rooting of softwood cuttings. Hartmann et al. (3) proposed that increased rooting associated with shoot tips, which include a terminal bud, may be due to increased concentrations of endogenous root promoters produced in the terminal bud. In addition, cells within the most recently developed tissues may not be fully differentiated and could become meristematic. Averaged across IBA concentrations, 81% of distal hardwood cuttings rooted, with 56% and 37% rooting of the semi-hardwood and softwood distal halves,

Table 1. Effect of cutting type on overall percent rooting of stem cuttings of 'Carolina Sapphire' smooth Arizona cypress taken at three growth stages and averaged across all IBA concentrations.

	Growth stage		
Cutting type	Semi-hardwood	Hardwood	Softwood
Terminal 30 cm			
Entire	31.7c[z]	63.9b	34.3a
Distal half	56.1a	80.5a	37.0a
Proximal half	45.0b	64.4b	26.9a

[z]Mean separation within columns by LSD, P = 0.05.

Table 2. **Effect of IBA concentration, by cutting type, on percent rooting of hardwood cuttings of 'Carolina Sapphire' smooth Arizona cypress.**

IBA concn. (ppm)	Cutting type (terminal 30 cm)			
	Entire	Distal half	Proximal half	Across cutting type
0	61.1ab[z]	69.4a	41.7b	57.4
4,000	61.1a	75.0a	72.2a	69.4
8,000	61.1a	83.3a	63.9a	69.4
12,000	66.7a	86.1a	69.4a	74.0
16,000	69.4a	88.9a	75.0a	77.8
Linear	NS	*	*	**
Quadratic	NS	NS	NS	NS
IBA vs. control[y]	NS	NS	*	*

[z]Mean separation within rows by LSD, P = 0.05.

[y]Linear contrast.

NS, *, ** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. **Effect of IBA concentration, by cutting type, on percent rooting of semi-hardwood cuttings of 'Carolina Sapphire' smooth Arizona cypress.**

IBA concn. (ppm)	Cutting type (terminal 30 cm)			
	Entire	Distal half	Proximal half	Across cutting type
0	11.1ab[z]	30.6a	8.3b	16.7
4,000	19.4b	61.1a	38.9ab	39.8
8,000	27.8b	61.1a	50.0ab	46.3
12,000	50.0a	55.6a	52.8a	52.8
16,000	50.0a	72.2a	75.0a	65.7
Linear	**	**	***	***
Quadratic	NS	NS	NS	NS
IBA vs. control[y]	*	*	*	*

[z]Mean separation within rows by LSD, P = 0.05.

[y]Linear contrast.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or .001, respectively.

respectively (Table 1). Softwood cuttings showed no significant difference among cutting types. Poor rooting of the softwood cuttings is similar to findings for several other coniferous species (3, 4).

For distal and proximal halves of hardwood cuttings, there was a linear relationship between percent rooting and IBA concentration (Table 2). At 16,000 ppm (1.6%) IBA, distal halves and proximal halves rooted at 89% and 75%, respectively. Entire terminal cuttings, however, showed no significant rooting response to IBA. On the other hand, rooting of semi-hardwood cuttings exhibited a linear relationship between all cutting types and IBA concentrations (Table 3). However, the data indicate that proximal and distal halves had a greater capacity for adventitious rooting compared with entire terminals. For softwood cuttings, rooting response was averaged across cutting type, and a quadratic response was noted for IBA treatment (Table 4). Treatment with IBA inhibited rooting, possibly because the softwood cuttings contained sufficient levels of root promoting substances, such that additional auxin was unnecessary and even inhibitory.

Rooting percentages were used to judge the degree of rooting whereas root count, total root length, total root area, and root dry weight data were recorded to judge quality of the rooted cuttings. Root count varied considerably among growth stages. As mentioned previously, at the hardwood growth stage there was a significant interaction ($P \leq 0.01$) between cutting type and IBA (Table 5). Effect of IBA was dependent upon cutting type. Distal halves and entire terminals were not affected significantly by IBA, while proximal halves displayed a linear response to IBA. The linear response for proximal halves only, and consequently the interaction, appeared to be due to the high mean root count of 7.3 roots at 16,000 ppm (1.6%) IBA for the proximal cuttings, which may be an aberrant figure. Jull et al. (5) found a similar interaction with 'Yoshino' cryptomeria [*Cryptomeria japonica* (L.f.) D. Don 'Yoshino'].

At the semi-hardwood stage, IBA and cutting type had significant influences on mean root count (Table 6). Root counts of all cutting types increased linearly with IBA concentration with mean root counts ranging from 3.7 to 9.8 for cuttings treated with 16,000 ppm (1.6%) IBA. In contrast to rooting percentages, distal halves had the fewest roots, whereas, entire terminals and proximal halves had more roots overall, i.e., averaged across IBA concentrations.

Table 4. **Effect of IBA concentration, across cutting type, on percent rooting and root count of softwood cuttings of 'Carolina Sapphire' smooth Arizona cypress.[z]**

IBA concn. (ppm)	Rooting (%)	Root count
0	53.7	1.5
2,000	35.1	2.4
4,000	31.5	1.8
8,000	25.9	3.4
12,000	25.9	5.5
16,000	24.1	4.1
Linear	**	**
Quadratic	*	NS
IBA vs. control[y]	*	*

[z]Data represent three replications.

[y]Linear contrast.

NS, *, ** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 5. **Effect of IBA concentration, by cutting type, on root count of hardwood cuttings of 'Carolina Sapphire' smooth Arizona cypress.**

IBA concn. (ppm)	Cutting type (terminal 30 cm)		
	Entire	Distal half	Proximal half
0	2.6	3.3	2.3
4,000	4.2	2.8	4.0
8,000	3.0	5.3	3.5
12,000	3.9	4.3	3.3
16,000	4.1	3.7	7.3
Linear	NS	NS	**
Quadratic	NS	NS	NS
IBA vs. control[z]	NS	NS	*

[z]Linear contrast.

NS, *, **, Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 6. **Effect of IBA concentration, by cutting type, on mean root count of semi-hardwood cuttings of 'Carolina Sapphire' smooth Arizona cypress.**

IBA concn. (ppm)	Cutting type (terminal 30 cm) Entire	Distal half	Proximal half	Across cutting type
0	1.2a[z]	2.0a	2.5a	1.9
4,000	1.8a	2.4a	3.4a	2.7
8,000	7.1a	3.9a	6.0a	5.7
12,000	7.1a	4.1a	5.5a	5.6
16,000	9.8a	3.7b	8.2a	7.2
Linear	*	**	**	***
Quadratic	NS	NS	NS	NS
IBA vs. control[y]	*	NS	*	*

[z]Mean separation within rows by LSD, P = 0.05.

[y]Linear contrast.

NS, *, **, *** Nonsignificant or significant at P ≤ 0.05, 0.01 or 0.001, respectively.

At the softwood stage, cutting type was nonsignificant in the ANOVA, and therefore, when averaged across cutting type, root counts increased linearly with IBA (Table 4). Overall mean root counts ranged from 1.5 to 5.5.

At the semi-hardwood and hardwood growth stages, total root length, total root area, and root dry weight were highly correlated (P ≤ 0.0001, r = 0.87). Thus, only mean root dry weights were recorded for the softwood cuttings and, only root dry weight data for the three growth stages will be discussed. For softwood and semi-hardwood cuttings root dry weight was not significantly affected by cutting type or IBA treatment. However, when averaged across cutting type, semi-hardwood cuttings displayed a linear response to IBA treatment (data not presented). Mean root dry weights for the semi-hardwood growth stage ranged from 15 mg to 37 mg when averaged across cutting type; softwood cuttings avaraged approximately 26 mg. Mean root dry weights for hardwood cuttings was unresponsive to IBA; however, cutting type was significant (data not presented) whereby entire terminals and proximal halves had significantly higher root dry weight than distal halves (93 and 85 mg vs. 50 mg).

Due to high mortality, data in Table 4 for the softwood cuttings only represent three replications of the treatments. Poor performance of the softwood cuttings was surprising since an unpublished study by the authors suggested softwood cuttings may have the potential to root in percentages comparable to semi-hardwood and hardwood cuttings. This related study resulted in 70% rooting of terminal 15 cm (6 in) softwood cuttings treated with 4,000 ppm (0.4%) IBA in 50% isopropanol.

The reason for reduced rooting of the softwood cuttings is unknown. However, Dirr (1), working with stem cuttings of *Cupressus arizonica* Greene (Arizona cypress), indicated poor rooting and rapid decline of cuttings due to excessive moisture. Although in the present study attempts were made to reduce the amount of water applied to the cuttings, excessive moisture may have been a contributing factor in addition to possible attack by microorganisms.

Our results indicate that stem cuttings of 'Carolina Sapphire' smooth Arizona cypress can be rooted at any growth stage. Rooting percentages exceeding 70% can be achieved using semi-hardwood or hardwood distal and proximal halves of 30 cm (12 in) terminal cuttings and treating the cuttings with 16,000 ppm (1.6%) IBA. At the hardwood stage 30 cm (12 in) terminals will root at approximately 70%, but higher rooting percentages can be achieved using distal and proximal halves. On the other hand, softwood cuttings did not root as well, with moderate rooting of 54% for nontreated cuttings. Highest root counts were recorded for semi-hardwood, entire terminals and proximal halves with root counts exceeding 8.0 at 16,000 ppm (1.6%) IBA. Root count for distal halves during the semi-hardwood growth stage was approximately 4.0, with similar root counts exhibited for all cutting types during the hardwood and softwood growth stages.

Although rooting of stem cuttings of 'Carolina Sapphire' smooth Arizona cypress is possible at all growth stages, it appears softwood cuttings are more difficult to root. However, with proper management of the rooting environment it may be possible to achieve rooting of softwood cuttings at percentages similar to those of semi-hardwood or hardwood cuttings. Difficulties encountered when rooting softwood cuttings suggest that propagators should root cuttings during the semi-hardwood or hardwood stages.

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