SECTION 8

Propagation

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Section 1 and Section 13 may contain related titles.

Stock Plant Nitrogen Status Affects Survival, Growth, and Adventitious Rooting of Stem Cuttings of Loblolly Pine

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Nature of Work: Many factors influence adventitious root formation one of which is genetics (1). Furthermore, although precise relationships between nitrogen and adventitious rooting have not been established, rooting success can be manipulated by varying N fertilization provided to stock plants. Henry et al. (2) reported optimal growth of eastem redcedar (*Juniperus virginiana* L.) when stock plants were provided weekly with 180 ppm N, but optimal rooting of stem cuttings occurred at only 20 ppm N. Rowe et al. (3) also observed decreased rooting at higher tissue N concentrations in cuttings of loblolly pine (*Pinus taeda* L.). However, little research has been conducted with pines (*Pinus* L. spp.) regarding the influence of stock plant N fertility on changes in tissue N content and root formation over the course of rooting under intermittent mist. Therefore, our objective was to detemmine if selected levels of applied nitrogen supplied to two families of hedged stock plants of loblolly pine would influence survival, growth, and adventitious rooting with respect to nitrogen content of the cuttings taken from those stock plants. In addition, we wanted to quantify changes in N status of stem cuttings during rooting under intermittent mist.

Hedged stock plants cultured in 11.4 L (3-gal) pots were arranged in a randomized complete block design with four blocks each containing two full-sib composite families (controlled pollinations where both parents were known) designated BR and GW and five N treatments. From previous work it was determined that composite family BR was composed of poor rooting families (< 10%) and composite family GW was composed of good rooters (> 40%). Trees for the five N treatments were grown in a medium of 6 perlite: 4 sand (v/v). The N treatments consisted of five levels of N (10, 25, 40, 55, or 70 ppm N) provided daily as NH_4NO_3 through an automated irrigation system. All other mineral nutrients were supplied at optimal levels.

During May 1995, 9 cm (3.5 in) long softwood terminal stem cuttings were taken from the hedged stock plants for tissue analysis and rooting experiments. One set of cuttings was analyzed for N with a CHN elemental analyzer. The other set was utilized for rooting experiments with cuttings inserted into flats containing a medium of 1 perlite: 1 coarse vermiculite (vie) and placed in a greenhouse under intermittent mist. The cuttings were not treated with auxin.

At 3, 6, 9, and 12 weeks after sticking, 15 cuttings from each family/N treatment were evaluated for survival, rooting, height, top dry weight, stem diameter measured 1 cm above the base, root count, and total root length. A cutting having at least one primary root ≥1 mm in length was considered rooted. Samples were Iyophilized to determine dry weights and were processed for N analysis as described previoualy. Means for each group of 15 cuttings were subjected to analysis of variance (ANOVA) procedures to detemmine aignificant main effects and interactions due to families and applied N levels.

Results and Discussion: Rooting was not observed until 6 weeks after cuttings were placed into the rooting medium and the majority of cunings which rooted formed roots by week 9 (Fig. 1A). By week 12, percent rooting for those cuttings taken from atock plants receiving N at 25 to 70 mg L^{-1} ranged from 28.3% to 33.29%, whereas only 17.0% of the cuttings from plants receiving 10 mg.L^{-1} had rooted. The opposite was true for the percentage of cuttings that remained alive during the 12 week rooting period. By week 12, 98.3% of the cuttings taken from stock plants receiving N at 10 mg.L^{-1} were still alive, while only 81.5% and 81.7% of the more succulent cuttings receiving 55 and 70 mg L-^{1}, respectively, were alive (Fig. 1B).

Nearly all increases in cutting height occurred within the first 3 weeks (Fig. 1 C). In contrast, top dry weight increased steadily throughout the experiment (Fig. 1 D). Although all cutlings were 90 mm (3.5 in) in length when inserted into the rooting medium, those cuttings taken from stock plants receiving higher rates of N had greater dry weights at week 0 and continued to have greater dry weights over the entire 12 weeks. However, regardless of applied N rate, the rate of increase in dry weight was sirrJbr for dl treatments. Stem diameters increased up to week 3, but thon decreased for the remainder of the rooting period (Fig.1E). Decreases in stern diameters may have rosulted from 9 gradual tissue desiccation. Similar to top dry weights, cuttings taken from stock plants receiving the higher rates of applied N had greater stem diameter. However, there was no correlation between rooting and stem diameters. As expected, total root length per cutting increased over time (Fig. 1F). There was no discernble pattern among N treatments, although by week 12 cuttings taken from stock plants receiving N at 55 mg.L^{-1} had the greatest total root length. Few additional roots formed after week 6 (Fig. 1G). In regards to tissue analysis, N contents were greater in cuttings from stock plants receiving the higher applied rates of N (data not presented) and these cuttings maintained higher levels throughout the 12 week rooting period.

There were no significant differences in rooting between the two composite families until week 12, where 32% of cuttings from family GW had rooted in comparison to family BR with only 24% rooting (data not presented). The poor rooting families (family BR) also remained alive in greater numbers than the good rooting family (family GW) (data not presented). By week 12, 94.3% of the cuttings of family BR were still alive in comparison to family GW where only 81.9% were viable. Where sign)ficant differences existed between the two composite families, family GW contained greater quantities of N (data not presented).

Significance to Industry: Stock plant N fertility influenced initial tissue N statw and subsequent survival and adventitious rooting of stem cuttings of loblolly pine. Cuttings removed from stock plants receiving tho lowest N fertilization rate rooted in the lowest percentages, but had higher survival rates. Furthermore, genetic variation played an important role as families responded differently to varying applied N levels. Cultings of the good rooting composite family rooted in higher percentages than those of the poor rooting composite family, but cuttings of the poor rooting family remained alive in greater numbers. Similar results may be expected for other woody species.

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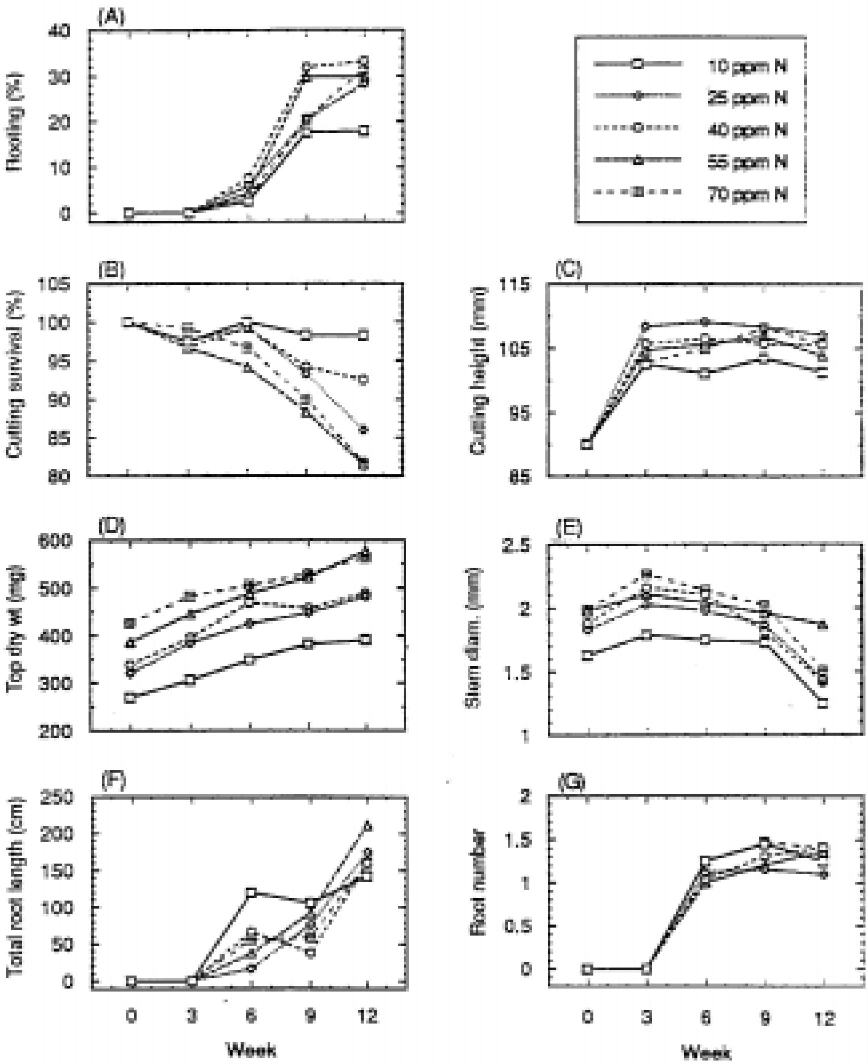

Figure 1. Effect of stock plant nitrogen fertilization on (A) rooting percentage; (B) cutting survival; (C) cutting height; (D) top dry weight; (E) total root length, and (G) number of roots per rooted cutting of stem cutting of loblolly pine placed under intermittent mist for 12 weeks. Cuttings were taken initially from hedged stock plants receiving N at either 10,25,40,55, or 70 mg.L^{-1}. Each symbol is based on eight means. Data are averaged over families.

Propagation of *Thuja* x 'Green Giant' by Hardwood Cuttings

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Nature of Work: *Thuja* x 'Green Giant' (syn. T. 'Giganteoides') is a hybrid of *Thuja plicata* J. Donn ex D. Don (western red cedar) and *Thuja standishii* (Gord.) Carrière (Japanese arborvitae) (Kim Tripp, personal communication). The cultivar exhibits a rapid growth rate, approaching 1 m (3.3 ft) of height growth per year, while maintaining a tightly pyramidal habit. Summer foliage is a lustrous dark green turning a shade of bronze in winter where exposed to sunlight. At maturity, 'Green Giant' can reach a height of 18 m (60 ft) without sacrificing superior growth form (1). 'Green Giant' is reported to be hardy to USDA Zone 5 and tolerant of stressful landscapes and clay soils (1). Trees do not appear to be susceptible to wind throw and significant pest problems have not been observed. These qualities make 'Green Giant' a promising landscape plant with utility as a fast growing hedge. To date, an extensive literature search has failed to reveal any information on propagation of *T.* x 'Green Giant'. Therefore, the objective of this research was to develop a protocol for successful propagation of this cultivar by hardwood stem cuttings.

On February 15, 1997, fifty terminal cuttings, approximately 45 cm (18 in) in length, were collected from the lower 2 m (6.5 ft) of each of seven trees growing under uniform fertility near Boonville, NC. Cuttings were packed on ice and transported to the Horticultural Science Greenhouses, Raleigh, NC. From the initial cutting material, two types of cuttings were prepared: a 24 cm (9.5 in) terminal and a 20 cm (7.9 in) lateral cutting. A lateral cutting consisted of a side shoot removed from that portion of a terminal cutting which was inserted in the rooting medium. The basal 1 cm (0.4 in) of all cuttings were then treated for 1 sec with 0, 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%) indolebutyric acid (IBA) in 50% isopropanol. The cuttings were air dried for 15 min before inserting the basal 4 cm (1.6 in) into a raised green house bench containing a medium of 2 perlite : 1 peat (v/v) with bottom heat maintained at 24° (2°C (75° (4°F). Intermittent mist operated daily for 5 sec every 5 min from 7:30 am to 6:00 pm. Cuttings were maintained under natural photoperiod and irradiance with day/nights of 24° ± 5°C (75° ± 9°F)/ 18° ± 5°C (65° ± 9°F). The experimental design (within the rooting bench) was a randomized complete block with a factorial arrangement of treatments (two cutting types x four IBA levels), six blocks, and six cuttings per treatment per block. After 6 weeks, cuttings were harvested and data recorded. Data included, percent rooting, number of primary roots ≥ 1 mm (0.04 in), and root lengths. All data except rooting percentages were based on the actual number of cuttings that rooted (at least one primary root). Data were subjected to analysis of variance and regression analysis.

Results and Discussion: Auxin treatment had no significant affect on percent rooting with rooting percentages of both terminal and lateral cuttings ranging from 93% to 100%. Averaged over all IBA treatments, rooting percentages of terminal and lateral cuttings were identical (96%). Similarly, the type of cutting had no affect on root number with each averaging 11 roots per cutting. However, IBA treatment had a significant quadratic affect on root number with the greatest number of roots (14 per cutting) occurring following treatment with 6000 (0.6%) or 9000 ppm (0.9%) IBA.

With regards to root length, there was a significant interaction between cutting type and IBA concentration. Average root length for lateral cuttings was not influenced by IBA concentration [30 mm (1.1 in)]. On the other hand, root length of terminal cuttings responded to IBA treatment in a quadratic manner. Average root length of nontreated terminal cuttings was significantly shorter than the lateral counterparts [18 mm (0.7 in) vs. 31 mm (1.2 in) respectively]. However, at IBA concentrations ≥ 3000 ppm (0.3%), root lengths of terminal and lateral cuttings were virtually identical [≈ 32 mm (1.3 in)].

Significance to Industry: Results demonstrate that rapid and efficient propagation of *T.* x ‘Green Giant’ by hardwood cuttings is possible. Although percent rooting was not enhanced statistically by IBA treatment, quality of the rooted cuttings (root number) increased with IBA treatment. Treatment of terminal and lateral cuttings with 6000 ppm (0.6%) IBA not only resulted in 100% rooting, but also stimulated an average of 14 roots per cutting with an average root length of 31 mm (1.2 in). The fact that terminal and lateral cuttings root in high percentages should permit propagators to make efficient use of propagation material. The ease and speed or rooting suggest ‘Green Giant’ is an excellent candidate for ‘direct sticking’ as it’s rapid growth rate will ensure a salable plant in a very short period of time.

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Controlled Pollination of Flowering Dogwoods Using Honeybees

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Nature of work: Tennessee State University and the University of Tennessee are collaborating to develop new cultivars of flowering dogwood, *Cornus florida* L. (1). The overall objective of our work is to breed resistance to dogwood anthracnose caused by the fungus *Discula destructiva* Redlin in flowering dogwood. Although *C. florida* is generally very susceptible to dogwood anthracnose, resistance has been reported in some seedlings that survived a disease epidemic in Maryland (3). The resulting cultivars 'Presidential' and 'Santamour' may represent the best native sources of resistance to this disease (2). Specific objectives of the work reported herein were: 1) To investigate the extent to which cross-pollination occurs among the most commonly grown commercial cultivars; 2) To determine if Italian honeybees (*Apis millifera* L.) could be used to efficiently produce hybrids; and 3) To determine if the use of a bee attractant and a sugar solution would increase the efficiency of honeybee-mediated crosses.

Mature clones of 'Presidential' and 'Santamour' were used as pollen parents in hand-pollinated crosses to five commercial cultivars (Table 1). All trees used in these experiments were grown in 22 gallons containers with Morton's Nursery Mix (3 pine bark:1 builder's sand:1 peat moss) and maintained outdoors under 60% shade. Freshly collected pollen was used to hand pollinate all florets in flowers that were previously emasculated and covered with paper bags. Pollen was transferred to the receptive stigmatic surface of each pistil with either a glass microscope slide, a glass rod, or directly from one whole inflorescence to another. The bags were then replaced over the pollinated heads and were not removed until after the native dogwoods finished blooming. Possible incompatibility between cultivars was examined in a complete diallel cross of seven clones (Table 2) using the same hand-pollination techniques.

Honeybee-mediated crosses were conducted in the 1996 and 1997 breeding seasons. Trees were isolated in 8'x8'x10' enclosures covered with 60% polypropylene shade cloth prior to the blooming period of native dogwoods and experimental trees. Each of 16 enclosures contained two trees (one each of the cultivar 'Cloud 9' and 'Cherokee Chief') and a beehive containing approximately 5,000 young honeybees. The experiment was repeated in 1997 with the same trees. The onset of bloom was delayed in 1997 by maintaining trees in a refrigerated trailer from March 15 to May 15 to further reduce the possibility of pollen contamination from native dogwoods. The treatments listed in Tables 3 and 4 were applied one day after the bees were introduced.

Results and Discussion: Hand pollinated crosses. Table 1 reports the fruit set data for hand pollination in the dogwood anthracnose resistance-breeding program. The data shown include the number of flower heads, which bore fruits in the fall of '96. The average fruit production percentage was 44 % but ranged from 0 (no fruit) for 'Ozark Spring' x 'Presidential' to 100% (all flower heads with at least one berry) for 'Cloud 9' x 'Santamour'.

Incompatibility and Self-fertility. Fruit-set data for the 7x7 complete diallel cross is shown in Table 2. For each cross, the top number in the table indicates the number of flower heads that produced fruits and the lower number indicates the number of flower heads involved in controlled pollination. Examination of data for reciprocal crosses show no evidence for incompatibility between cultivars.

Honeybee-mediated Crosses. Results of the honeybee breeding experiment are reported in Tables 3 (1996) and 4 (1997). Flower and fruit data for both years are shown. For the 1996 breeding season, fruit harvest data is shown while for 1997 only mid-season fruit set data is recorded. The most important factor for fruit set appears to be the presence of honeybees. Some fruit set occurred in the no-bee treatments, which is attributed to the presence of other insects in the cages (Tables 3, 4). The highest fruit set in 1996 was for the treatment combining honeybees and a chemical attractant (Table 3). In 1997, the use of chemical attractant did not seem to influence fruit set. This is probably due to the higher flower number in 1997 than in 1996, providing less incentive for bees to forage.

Significance to Industry: Results form the present study suggest that new anthracnose-resistant dogwood cultivars can be developed using sources of resistance that have been identified The anthracnose resistant cultivars 'Presidential' and 'Santamour' were used successfully as pollen parents in hand-pollinated crosses with five commercial cultivars and hybrid seedlings were produced. No barrier to sexual compatibility among the cultivars tested was detected. This study demonstrates that honeybees can be used to efficiently cross *C. florida* cultivars. This is a labor saving method as opposed to traditional hand breeding methods. Several thousand hybrids produced through this project are being evaluated for disease resistance by the University of Tennessee. Evaluation of these hybrids could result in the release of commercially valuable anthracnose resistant selections.

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Table 1. Harvested fruit data for hand pollinated crosses in 1996 dogwood anthracnose breeding.

	Number of flower heads		
Cross (♀ x ♂)	Pollinated	With fruit	Fruit (%)[a]
Cherokee Brave x Presidential	73	11	15
Cherokee Brave x Santamour	52	13	25
Cherokee Chief x Presidential	70	59	84
Cherokee Chief x Santamour	72	48	67
Cloud 9 x Presidential	20	12	60
Cloud 9 x Santamour	13	13	100
Cherokee Princess x Presidential	12	2	17
Ozark Spring x Presidential	3	0	0
Ozark Spring x Santamour	50	1	2

[a] Number of flower heads with fruit expressed as percentage of number of flower heads pollinated.

Table 2. Diallel matrix showing fruit set (number of heads harvested in the numerator) per number of controlled pollinations (number of heads pollinated in the denominator) on seven *Cornus florida* cultivars.

♀ / ♂	CB	CC	CP	CL9	OS	RB	YB
Cherokee Brave (CB)	0/7	1/4	0/5	0/7	0/4	2/3	0/4
Cherokee Chief (CC)	11/12	3/6	4/6	5/5	3/4	4/6	4/5
Cherokee Princess (CP)	1/6	3/8	0/4	0/7	5/10	0/0	4/8
Cloud 9 (CL9)	10/28	6/13	12/18	6/17	7/14	3/9	7/16
Ozark Spring (OS)	1/10	0/4	0/5	3/4	2/4	3/4	3/4
Red Beauty (RB)	4/5	3/3	2/3	1/4	0/3	0/0	1/2
Yellowberry (YB)	5/7	2/4	2/4	3/4	3/4	2/4	2/6

Table 3. Late-season 1996 harvested fruit data for honeybee mediated pollination of two *Cornus florida* cultivars: ‘Cloud 9’ and Cherokee Chief’.

Treatment	Total (flower buds)	With fruit set	Fruit set (%)[a]
Attractant[b]	350	12	3.4
Attractant + sugar	422	1	0.02
Attractant + bees	400	50	12.5
Attractant + sugar + bees	231	20	8.7
Sugar[c]	288	25	8.7
Sugar + bees	168	15	8.9
Bees	201	12	5.9
Control	594	2	0.03

[a]Final fruit set is calculated as the number of mature fruit harvested expressed as a percentage of the number of flower heads.
[b]Chemical attractant (queen mandibular pheromone) PheroTech , Delta, BC, Canada.
[c]10% sugar solution.

Table 4. Mid-season 1997 fruit set data) for honeybee mediated pollination of two Cornus florida cultivars: ‘Cloud 9’ and Cherokee Chief’.

Treatment	Total (flower buds)	With fruit set	Fruit set (%)[a]
Attractant[b]	2025	8	0.4
Attractant + sugar	2296	26	1.1
Attractant + bees	1445	540	37.4
Attractant + sugar + bees	1522	504	33.0
Sugar[c]	1978	155	8.0
Sugar + bees	1801	677	38.0
Bees	1890	682	36.0
Control	1640	55	3.3

[a]Fruit set is calculated as the number of fruit set expressed as a percentage of the number of flower heads.
[b]Chemical attractant (queen mandibular pheromone) PheroTech , Delta, BC, Cana[c]10% sugar solution