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Mineral Nutrient and Carbohydrate Status of Loblolly Pine during Mist Propagation as Influenced by Stock Plant Nitrogen Fertility

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Abstract. **Hedged stock plants of four full-sib families [27-2 x 27-5, 27-3 x 27-1, 27-2 x 27-1, and 27-6 x 27-1 (designated B, G, R, and W)] of loblolly pine (*Pinus taeda* L.) were fertilized daily with a complete nutrient solution containing N at 10, 25, 40, 55, or 70 mg·L^{-1}. In May, terminal softwood stem cuttings were taken and placed under intermittent mist. Families were combined to form composite poor-rooting (BR) and good-rooting (GW) families. At 0, 3, 6, 9, and 12 weeks after sticking, cuttings were evaluated for rooting and analyzed for mineral nutrient and carbohydrate content. Percent rooting by week 12 for cuttings from stock plants receiving N between 25 to 70 mg·L^{-1} was 28% to 33%, whereas significantly fewer (17%) cuttings from plants receiving 10 mg·L^{-1} had rooted. By week 12, 98% of cuttings taken from stock plants receiving N at 10 mg·L^{-1} were alive, while significantly fewer (81% and 82%) of the more succulent cuttings receiving 55 and 70 mg·L^{-1}, respectively, had survived. Nearly all increases in cutting height occurred within the first 3 weeks. In contrast, top dry weight increased steadily throughout the experiment. There were no significant differences in rooting between the two composite families until week 12, when 32% of cuttings from family GW had rooted compared with 24% for family BR. Survival of cuttings was greater for the poor-rooting family (BR) (94%) than for the good-rooting family (GW) (82%) after 12 weeks. Levels of total nonstructural carbohydrates (TNC) and individual soluble sugars were initially higher in cuttings taken from stock plants that received higher rates of N, whereas the reverse was true for starch content. With the exception of sucrose, content of TNC and soluble carbohydrates generally increased over time. Starch was nearly depleted by week 3, but had increased by weeks 6 and 9. No correlation was found between TNC : N ratios and rooting percentage. Family GW contained greater quantities of myo-inositol, glucose, fructose, sucrose, total soluble carbohydrates (TSC), and TNC than did family BR. Mineral nutrient content was generally greater in cuttings taken from stock plants that received higher rates of N; these cuttings also maintained higher levels throughout the 12-week rooting period. As with the soluble carbohydrates, the good-rooting composite family (GW) contained greater amounts of all mineral nutrients than did the poor-rooting family BR.**

Loblolly pine is one of the fastest growing pines and is the leading commercial timber species in the southeastern United States (Allen et al., 1990). Nearly all loblolly pine is propagated currently by seed, which results in considerable undesirable genetic variation among trees (Zobel and Talbert, 1991). Development of techniques for vegetative propagation would reduce this variability and permit cloning of elite trees. Recently, considerable research has been conducted to develop micropropagation protocols for the species. Although some success has been achieved, current protocols do not permit large-scale propagation of elite trees (Gupta et al., 1993; Handley et al., 1995). Therefore, because of limitations of both seed and tissue culture propagation, there is currently much interest in vegetative propagation by stem cuttings.

Success in rooting is partially dependent upon carbohydrate and mineral nutrient content of cuttings, in particular the carbohydrate to nitrogen ratio. Carbohydrate content of stem cuttings during rooting has been studied for several species, but whether or not carbohydrates directly influence adventitious root formation is still unclear (Haissig, 1986, 1989; Veierskov, 1988). Furthermore, various mineral nutrients are required for root growth and development (Blazich, 1988) and can influence rooting (Henry et al., 1992; Welander, 1995).

Changes in mineral nutrient and carbohydrate status over the course of rooting under intermittent mist have been described for jack pine (*Pinus banksiana* Lamb.) (Haissig, 1989, 1990), Scots pine (*Pinus sylvestris* L.) (Ernstsen and Hansen, 1986; Hansen et al., 1978), and radiata pine (*Pinus radiata* D. Don.) (Cameron and Rook, 1973). However, no research of this type has been conducted with loblolly pine. Therefore, the objective of this study was to quantify changes in mineral nutrient and carbohydrate status of stem cuttings in two composite families of loblolly pine during intermittent mist propagation as influenced by stock plant N fertility.

Materials and Methods

Plant material and N application. Hedged stock plants of four full-sib families [27-2 x 27-5, 27-3 x 27-1, 27-2 x 27-1, and 27-6 x 27-1 (designated B, G, R, and W)] of loblolly pine were fertilized outdoors on a gravel container pad at a range of applied-N levels (Rowe, 1996). The treatments (within each of four blocks) were 1) four, full-sib families (controlled pollinations where both parents are known), and 2) five N treatments, arranged in a complete factorial with 24 treatments and 384 trees. Nitrogen (10, 25, 40, 55, or 70 mg·L^{-1}) was supplied daily as NH_4NO_3 through an automated irrigation system. Each block-family–N combination consisted of a four-tree-row plot as the experimental unit. Results of a preliminary study demonstrated that cuttings from families B and R rooted poorly (<10%) whereas cuttings from families G and W rooted well (>40%). For this reason, cuttings were combined for families B and R [poor-rooting family (BR)] and families G and W [good-rooting family (GW)].

Carbohydrate analysis. Terminal softwood stem cuttings (9 cm long) were collected in May from orthotropic shoots of hedged stock plants. One set of cuttings was analyzed to determine initial carbohydrate and mineral nutrient status. Carbohydrates were determined by high-performance liquid chromatography (soluble carbohydrates) or enzymatic analysis (starch). Lyophilized samples were extracted four times in 80% ethanol and centrifuged at 13,800 g_n for 10 min following each extraction. The decanted supernatant was evaporated, resolubulized in deionized H_2O, and

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centrifuged through microfilter columns packed with 200 µL Serdolit Red Micro (H^+-form) cation exchange resin (Serva Feinbiochemica, Heidelberg, Germany), 400 µL polyvinylpolypyrrolidone (PVPP), and 600 µL Serdolit Blue Micro (HCO_3^--form) anion exchange resin to remove charged particles such as organic acids, amino acids, inorganic ions, phosphate esters, and phenolic compounds. Samples were then analyzed for glucose, fructose, sucrose, raffinose, and the sugar alcohols myo-inositol and pinitol, utilizing a Dionex Series 4000i system (Dionex Corp., Sunnyvale, Calif.) for high-performance liquid chromatography, using a Carbo Pak PA-1 anion exchange column and a pulsed amperometric detector. Individual sugars were identified based on retention times relative to known standards. Since myo-inositol and pinitol were eluted at the same time, they were indistinguishable with the methods used and thus all carbohydrates eluted in this peak were considered to be myo-inositol.

The remaining insoluble pellet from the extraction process was utilized for enzymatic determination of starch. The pellet was resuspended in 1.5 mL 30 mM HCl and boiled for 30 min. After the hydrolysate had cooled, pH was adjusted to 4.5 using 30 mM KOH. The gelatinized starch was digested for 60 min at 55 °C using 36 units amyloglucosidase [from *Aspergillus oryzae* (Ahlburg) Cohn.]. The amyloglucosidase had been dialyzed previously against 50 mM Na-acetate buffer (pH 4.5). Samples were boiled for 1 min to stop the reaction. After cooling and centrifugation, an aliquot of the supernatant was used to measure glucose in a 1-mL solution containing 100 mM Hepes-NaOH (pH 8.0), 5 mM $MgCl_2$, 1 mM NAD, 1 mM ATP, 5 mM DTT, 2.5 units hexokinase (from Bakers yeast), and 2.5 units glucose 6-P dehydrogenase [from *Leuconostoc mesenteroides* (Tsenkovskii) van Tieghem]. The reaction mixture was incubated at 25 °C for 30 min and its absorbance at 340 nm was measured on a Milton Roy 1201 spectrophotometer (Milton Roy, Rochester, N.Y.) to determine starch content.

Mineral nutrient analysis. For analysis of mineral nutrients, lyophilized tissue samples were digested in HNO_3 and the ash was dissolved in 10 mL concentrated HCl and diluted to 50 mL with deionized water. An aliquot from this solution was then analyzed for P, K, Ca, S, Mg, Mn, B, Fe, and Zn with a Perkin-Elmer Plasma 2000 inductively coupled plasma emission spectrometer (Perkin Elmer Corp., Norwalk, Conn.). Total N was determined by combusting 10-mg samples to elemental gases in a Perkin Elmer 2400 CHN elemental analyzer. All mineral nutrient analyses were conducted by the Analytical Service Laboratory, Dept. of Soil Science.

Rooting of cuttings. A second set of cuttings was utilized for rooting experiments conducted at the Horticultural Science Greenhouse, Raleigh. The proximal 2 cm of each cutting was inserted into a 15-cm-deep, raised greenhouse bench containing 1 peat : 1 perlite (v/v). Needles on the basal portions of cuttings were not removed and the cuttings were not treated with auxin. Intermittent mist was supplied daily for 6 to 8 s every 10 to 15 min from sunrise to sunset. Cuttings were maintained under natural photoperiod and irradiance with temperatures of 24 ± 5 °C day/16 ± 5 °C night.

The experimental design within the propagation bed was a split-plot with main plots (two composite families) arranged as a randomized complete block and subplots (five N rates) randomized within each main plot. There were four blocks and 60 cuttings (four rows of 15) within each treatment combination.

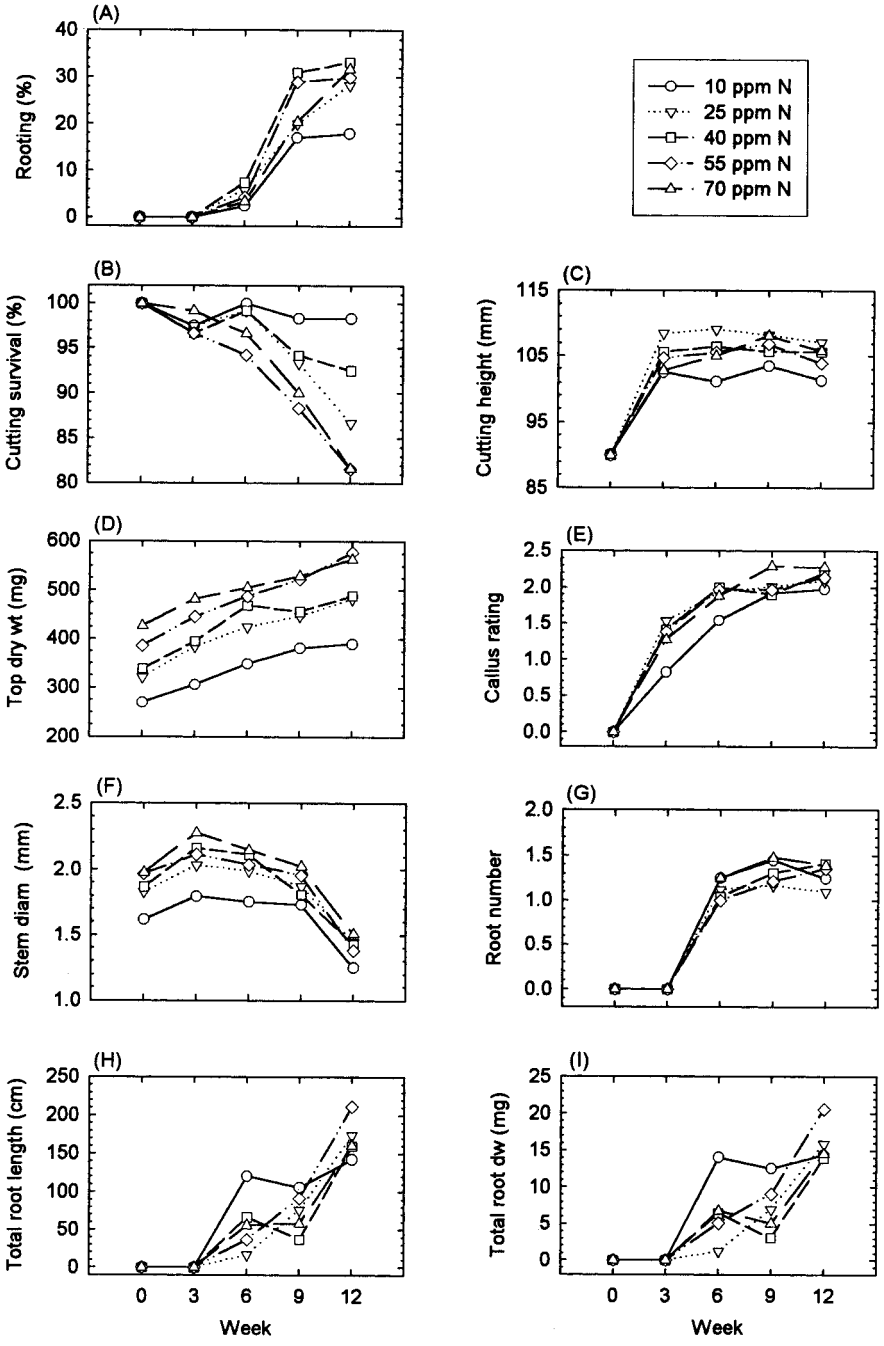

Fig. 1. Effect of stock plant nitrogen fertilization on (**A**) rooting percentage; (**B**) cutting survival; (**C**) cutting height; (**D**) top dry weight; (**E**) callus rating; (**F**) stem diameter; (**G**) number of roots per rooted cutting; (**H**) total root length per rooted cutting; and (**I**)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

Initially, cuttings were drenched with *N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (Captan 50 WP; Blue Ribbon Products Co., Devitt, N.Y.) at 2.4 g·L^{-1}. Cuttings were sprayed weekly for the remainder of the rooting period, alternating chlorothalonil [tetrachloroisophthalonitrile (Daconil 2787 Flowable, ISK Biotech Corp., Mentor, Ohio)] at 2.5 mL·L^{-1} and mancozeb, a coordination product of zinc ion, manganese, and ethylene biodithiocarbamate (Fore, Rohm and Haas, Philadelphia) at 1.2 g·L^{-1}.

At 3, 6, 9, and 12 weeks after sticking, 15 cuttings from each treatment combination were harvested from within each block. Each cutting was evaluated for survival, rooting, height, top dry weight, callus, stem diameter measured 1 cm above the base, root count, total root length, root area, and root dry weight. A cutting having at least one primary root ≥1 mm in length was considered rooted. Callus formation was rated on a scale from 0 to 3 where 0 = no callus formation, 1 = callus present but less than the diameter of the stem, 2 = callus present and greater than the diameter of the stem, and 3 = callus formation greater than twice the diameter of the stem. Root area was measured utilizing a Monochrome Agvision System 286 Image Analyzer (Decagon Devices, Pullman, Wash.). Samples were lyophilized to determine dry weights and were processed for carbohydrate and mineral nutrient analysis as described previously.

Statistical analysis. Means for each group of 15 cuttings were subjected to analysis of variance (ANOVA) procedures to determine significant main effects and interactions among families and applied-N levels (SAS Institute, 1990). Family means were separated by the least significant difference (LSD) test. Pearson correlation coefficients (Proc CORR) were generated to describe the strength of relationships among various dependent variables such as TNC : N ratios of cuttings and rooting characteristics.

Table 1. Significant sources of variation in ANOVA for growth responses, mineral nutrient content, and carbohydrate content in stem cuttings of loblolly pine.

Observation df:	Week 4	Family 1	Nitrogen 4	F × N 4	W × F 4	W × N 16	W × F × N 16
Rooting	***	NS	NS	*	**	*	NS
Survival	***	***	***	***	***	*	NS
Height	***	***	***	**	***	**	NS
Top dry wt	***	***	***	NS	NS	NS	NS
Callus rating	***	**	***	NS	***	***	NS
Stem diam	NS	NS	NS	NS	NS	NS	NS
Root no./cutting	**	NS	NS	NS	*	NS	NS
Root length	***	NS	NS	NS	NS	NS	NS
Root dry wt	***	NS	*	NS	NS	NS	NS
N	***	***	***	*	NS	**	NS
P	***	***	***	*	NS	NS	NS
K	***	***	***	NS	NS	**	NS
S	***	***	***	NS	NS	***	NS
Ca	***	***	***	NS	*	***	NS
Mg	***	***	***	NS	NS	NS	NS
Fe	***	***	***	**	NS	NS	NS
B	***	***	***	NS	**	**	NS
Cu	***	***	***	***	NS	*	NS
Myo-inositol	***	***	***	NS	NS	NS	NS
Glucose	***	***	***	NS	*	**	NS
Fructose	***	***	***	NS	NS	**	NS
Sucrose	***	***	***	**	**	***	NS
Starch	***	NS	***	NS	NS	NS	NS
TSC[z]	***	***	***	NS	NS	NS	NS
TNC[y]	***	***	***	NS	NS	NS	NS
TNC:N[x]	**	NS	***	NS	NS	*	NS

[z]TSC = Total soluble carbohydrates.
[y]TNC = Total nonstructural carbohydrates.
[x]TNC : N = TNC to nitrogen ratio.
[NS, *,**,***]Nonsignificant or significant at $P \leq 0.10$, 0.05, or 0.01, respectively.

Table 2. Rooting and growth characteristics of stem cuttings of loblolly pine as influenced by family and time. Data are averaged over stock plant N treatment. (Means based on 20 sets of 15 cuttings.)

Rooting characteristic	Family	Week 0	3	6	9	12
Rooting (%)	BR	0 a[z]	0 a	6.4 a	18.8 a	24.3 b
	GW	0 a	0 a	3.0 a	19.3 a	32.1 a
Survival (%)	BR	100 a	97 a	98 a	98 a	94 a
	GW	100 a	98 a	97 a	87 a	82 b
Height (mm)	BR	90 a	108 a	109 a	110 a	107 a
	GW	90 a	102 b	102 b	103 b	102 b
Top dry wt (mg)	BR	316 b	375 b	426 b	444 b	476 b
	GW	381 a	430 a	468 a	491 a	524 a
Callus rating[y]	BR	0 a	1.2 a	2.0 a	2.1 a	2.2 a
	GW	0 a	1.3 a	1.7 b	1.9 a	2.1 b
Stem diam (mm)	BR	---	2.0 a	2.1 a	1.9 a	1.4 a
	GW	---	2.1 a	2.0 b	1.8 b	1.3 a
Root no. per cutting	BR	0 a	0 a	1.1 b	1.4 a	1.4 a
	GW	0 a	0 a	1.2 a	1.2 a	1.2 a
Root length (cm)	BR	0 a	0 a	47.0 b	78.5 a	188.8 a
	GW	0 a	0 a	64.4 a	72.8 a	153.2 a
Root dry wt (mg)	BR	0 a	0 a	4.9 b	7.2 a	16.5 a
	GW	0 a	0 a	7.7 a	8.0 a	15.4 a

[z]Mean separation between families within weeks by LSD, $P \leq 0.05$.
[y]0 = no callus formation, 1 = callus present but less than the diameter of the stem, 2 = callus present and greater than the diameter of the stem, and 3 = callus formation greater than twice the diameter of the stem.

Results and Discussion

Cutting survival and rooting. Rooting was not observed until 6 weeks after cuttings were placed into the rooting medium. Most of the cuttings that eventually rooted had formed roots by week 9 (Fig. 1A). By week 12, 28% to 33% of the cuttings taken from stock plants receiving N at 25 to 70 mg·L^{-1} had rooted, whereas significantly fewer (17%) cuttings from stock plants receiving 10 mg·L^{-1} had rooted. Similar results were obtained when cuttings were collected later in the year (summer softwood and winter hardwood cuttings) (Rowe, 1996). In contrast, the higher rates of N were detrimental to cutting survival. By week 12, 98% of the cuttings taken from stock plants receiving N at 10 mg·L^{-1} were still alive, while significantly fewer (81% and 82%) of the more succulent cuttings receiving 55 and 70 mg·L^{-1}, respectively, survived (Fig. 1B). Preston et al. (1953) also reported greater losses when succulent stem cuttings of azalea (*Rhododendron* L.) were used, especially those taken from plants receiving high N levels.

Nearly all increases in cutting height occurred within 3 weeks after sticking (Fig. 1C), whereas top dry weight increased steadily throughout the experiment (Fig. 1D). Cuttings taken from stock plants that received higher rates of N had greater top dry weights at week 0 and continued to have greater dry weights over the entire 12 weeks. The amount of callus formed at the base of the cutting increased with time (Fig. 1E). Cuttings taken from stock plants receiving N at 10 mg·L^{-1} had formed the least callus by weeks 3 and 6, but there were no differences among N treatments by week 12. Stem diameters increased up to week 3, but then tended to decrease for the remainder of the rooting period (Fig. 1F), perhaps because of a gradual desiccation of tissues over time. As with top dry weights, stem diameter was greatest for cuttings taken from stock plants that received higher rates of applied N. However, there was no significant correlation between rooting percentage and either callus production or stem diameters. In contrast, callus formation was positively correlated with rooting of jack pine (Haissig, 1990).

The number of roots formed was not affected by N treatment (Fig. 1G). Although few additional roots formed after week 6, total root length per cutting increased over time (Fig. 1H). There was no set pattern among N treatments, although by week 12 cuttings taken from stock plants receiving N at 55 mg·L^{-1} had significantly greater total root length (Fig. 1H), total root dry weight (Fig. 1I), and root

area (data not presented) than all other N treatments.

There was no significant difference in rooting percentage between the two composite families (Table 1) until week 12, when the rooting percentage in family GW (32%) was significantly greater than that in family BR (24%) (Table 2). This resulted in a significant week × family interaction (Table 1). In contrast, by week 12, survival of cuttings of the poor-rooting family (BR) (94%) was significantly greater than that of the good-rooting family (GW) (82%) (Table 2). Again, a week × family interaction was significant. Cutting height for composite family BR was greater throughout the rooting period, but dry weights were lower (Table 2). In general, after week 6, family BR had greater callus formation and stem diameters, whereas family GW had greater root number, length, and dry weight (Table 2). This is not surprising as there is substantial evidence that rooting ability is genetically controlled (Haissig and Riemenschneider, 1988).

Mineral nutrient status. Throughout the 12-week rooting period, total content of the eight mineral nutrients measured was generally greatest in cuttings from stock plants receiving higher rates of N (Fig. 2). Increased N content is often associated with higher contents of other mineral nutrients (Mengel and Kirkby, 1987). Nitrogen and P content remained relatively stable over time (Fig. 2 A and B), whereas K and B decreased (Fig. 2 C and H). Levels of S, Ca, Mg, Fe, and Cu increased during the rooting period (Fig. 2 D, E, F, G, and I). Slight increases in S, Ca, Mg, and Cu at week 3, prior to any root formation, suggest that nutrients were absorbed from the rooting medium or from the water used for mist; thereafter, increased levels of these elements suggest absorption by newly formed roots. Experimental evidence suggests that N, P, Ca, Mn, and Zn are all important for root initiation, whereas N, P, K, Ca, and B are required for root growth and development (Blazich, 1988). Thus, nutrients originally present in cuttings are probably more important for root initiation.

Cuttings of GW contained greater amounts of all mineral nutrients than did those of BR (Table 3), and these higher levels were generally maintained over the course of the rooting period. Cuttings of GW contained significantly greater quantities of Fe, Mg, Mn, Zn, and Cu. A week × family interaction was significant for N, K, S, Ca, B, and Cu. Although statistically significant, these values may not be horticulturally significant to the plant as they are similar numerically. However, previous studies have demonstrated that Zn fertilization of stock plants of grape (*Vitis* sp. cv. Chasselas x Berlandieri 41 B) increased rooting percentages of cuttings (Samish and Spiegel, 1958). Zinc has been positively correlated with rooting, as it is required for production of the auxin precursor tryptophan (Goodwin and Mercer, 1983). In contrast, one might expect Mn to be negatively correlated with rooting. Manganese is thought to activate IAA oxidase, which destroys native auxin

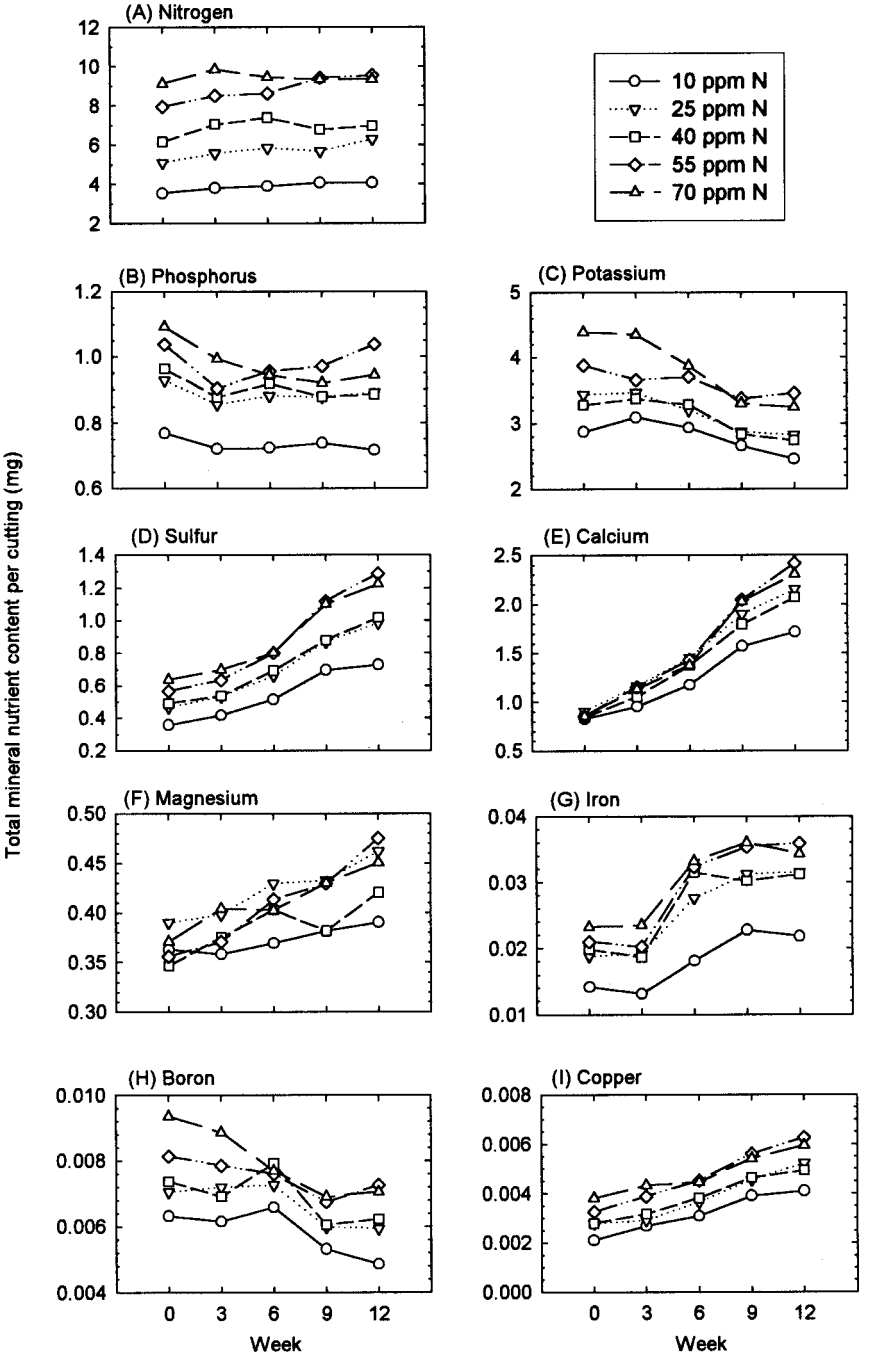

Fig. 2. Effect of stock plant nitrogen fertilization on mineral nutrient content of stem cuttings of loblolly pine taken from hedged stock plants during a 12-week rooting period under intermittent mist. (**A**) N; (**B**) P; (**C**) K; (**D**) S; (**E**) Ca; (**F**) Mg; (**G**) Fe; (**H**) B; and (**I**) Cu. Each symbol is based on eight means. Data are averaged over families.

(Tomaszewski and Thimann, 1966). In this study, cuttings of family GW initially contained greater quantities of Mn, but this difference had dissipated by week 3. Data for Mn and Zn content are not presented after week 3 because the cuttings were inadvertently sprayed with a fungicide containing these elements during week 4, rendering subsequent data unreliable. However, previous research with loblolly pine (Rowe, 1996) and avocado (*Persea americana* Mill.) (Reuveni and Raviv, 1981) revealed that Mn content was negatively correlated with rooting. High Mn levels were found in leaf tissue of cuttings taken from difficult-to-root avocado cultivars, whereas low levels were found in cuttings of easy-to-root cultivars. Thus, cuttings taken from plants with high concentrations of Mn might be more difficult to root.

Boron is required for root growth and development (Blazich, 1988) and family GW contained significantly greater amounts of B than did family BR. Henry et al. (1992) also found B to be positively correlated with rooting percentage, root count, root length, and root dry weight in eastern red cedar (*Juniperus virginiana* L.). In a previous study with loblolly pine, B appeared to influence rooting, despite

the fact that B and rooting were not highly correlated (Rowe, 1996); deficiencies of B in spring (7.0 mg·kg^{-1}) and summer (8.7 mg·kg^{-1}) control treatments, compared with the other five N treatments (21.7 mg·kg^{-1}), may have reduced rooting. Rooting percentages were only 0.8% in spring and 10% during summer, but increased to 61% for winter cuttings when tissue levels of B were restored to 15.9 mg·kg^{-1}. This occurred despite the fact that spring and summer control hedges produced the greatest number of shoots, contained high TNC levels, and exhibited no visible symptoms of mineral nutrient deficiency. Even though B at 7.0 mg·kg^{-1} is adequate for plant growth, higher amounts may promote adventitious rooting. Thus, further stimulation of rooting also may be possible by manipulation of stock plant B concentrations.

When considering the influence of mineral nutrients, one must address the relationships among elements and potential interactions. The possible promotive influence of Zn and the possible inhibitory influence of Mn may counteract one another. Zinc may have a stronger effect than Mn, thereby allowing family GW to exhibit slightly better rooting. Also, an individual element could promote or inhibit depending on its concentration. When additional elements are included, the potential for interactions further confounds the results and conclusions that can be drawn. Furthermore, when elements are antagonistic, individual correlations may be meaningless.

Carbohydrate status. Myo-inositol (Fig. 3A), glucose (Fig. 3B), fructose (Fig. 3C), sucrose (Fig. 3D), and total nonstructural carbohydrates (TNC) (Fig. 3F) were initially higher in cuttings taken from stock plants receiving higher rates of N. However, starch content was significantly lower (Fig. 3E). Stem cuttings must have an adequate supply of carbohydrates during root initiation (Bhattacharya et al., 1976; Borthwick et al., 1937). These carbohydrates may be present in the cutting at the time of severance or accumulated through photosynthesis during the rooting period. Shading cuttings of jack pine reduced both rooting and soluble sugar and starch content (Haissig, 1990), suggesting that carbohydrates produced after sticking were necessary for rooting.

Myo-inositol content increased after the cuttings were taken (Fig. 3A). Glucose (Fig. 3B), fructose (Fig. 3C), and TNC (Fig. 3F) content fluctuated over time and then increased sharply between weeks 9 and 12. In contrast, sucrose content declined sharply between weeks 9 and 12 (Fig. 3D). In leafy cuttings, TNC often accumulate during propagation, sometimes after an initial decrease (Haissig, 1990). Total carbohydrate content decreased initially in cuttings from mature trees of jack pine, but then increased in needles and terminal shoots. By the end of the propagation period, total carbohydrates in all tissues were less than at day 0 (Haissig, 1989), which is contrary to our results. In our study, levels fluctuated throughout the 12-week period, but the general trend was an increase in TNC. However, starch was nearly depleted by week 3 and never attained levels comparable to that present at week 0, except in those cuttings taken from stock plants that received N at 10 mg·L^{-1} (Fig. 3E).

Table 3. Mineral nutrient content (mg) in stem cuttings of loblolly pine as influenced by family and time. Data are averaged over stock plant N treatment. (Means based on 20 composite samples.)

Mineral nutrient	Family	Week 0	3	6	9	12
N	BR	5.7 b[z]	6.4 b	6.5 b	6.6 b	6.8 b
	GW	7.0 a	7.5 a	7.6 a	7.5 a	7.7 a
P	BR	0.88 b	0.80 b	0.82 b	0.81 b	0.83 b
	GW	1.04 a	0.94 a	0.95 a	0.94 a	0.96 a
K	BR	3.3 b	3.3 b	3.2 b	2.8 b	2.7 b
	GW	3.8 a	3.8 a	3.6 a	3.2 a	3.1 a
S	BR	0.46 b	0.53 b	0.67 b	0.88 b	0.98 b
	GW	0.55 a	0.60 a	0.72 a	0.99 a	1.12 a
Ca	BR	0.8 b	1.0 b	1.3 b	1.8 b	2.0 b
	GW	0.9 a	1.1 a	1.4 a	1.9 a	2.3 a
Mg	BR	0.35 b	0.36 b	0.39 a	0.40 b	0.42 b
	GW	0.38 a	0.40 a	0.41 a	0.42 a	0.46 a
Fe	BR	0.018 b	0.018 b	0.027 a	0.029 a	0.029 b
	GW	0.021 a	0.020 a	0.030 a	0.033 a	0.032 a
Mn	BR	0.056 b	0.055 a	---	---	---
	GW	0.067 a	0.056 a	---	---	---
Zn	BR	0.029 b	0.029 b	---	---	---
	GW	0.033 a	0.031 a	---	---	---
B	BR	0.007 b	0.007 b	0.007 a	0.006 b	0.006 a
	GW	0.008 a	0.008 a	0.008 a	0.007 a	0.007 a
Cu	BR	0.002 b	0.003 b	0.004 b	0.004 b	0.005 b
	GW	0.003 a	0.004 a	0.004 a	0.005 a	0.006 a

[z]Mean separation between families within each week by LSD, $P \leq 0.05$.

Carbohydrate levels present in the cutting at time of severance may be more important for rooting than carbohydrate accumulation. Net photosynthesis declined in cuttings of radiata pine (*Pinus radiata* D. Don), immediately after detachment, and remained low until roots had formed (Cameron and Rook, 1973), suggesting that photosynthesis provided little carbohydrate following severance. Gas exchange in most cuttings undergoes a steady decline following severance and does not increase until the onset of root formation (Davis, 1988; Smalley et al., 1991; Svenson et al., 1995). This may help explain the initial decrease in glucose, fructose, and TNC in cuttings taken from plants receiving high levels of applied N (Fig. 3 B, C, and F) and the subsequent increase in carbohydrates by week 6 when some rooting occurred. In contrast, leaf carbohydrate concentrations (glucose, sucrose, TSC, and TNC) in stem cuttings of red maple (*Acer rubrum* L. 'Red Sunset') increased until the onset of root formation and decreased thereafter, while changes in starch concentrations showed no clear pattern (Smalley et al., 1991). This would help explain the decrease in TNC at week 9 when the greatest increase in rooting occurred, since net loss of carbohydrate indicates use (Haissig, 1982).

The hypothesis that high TNC : N ratios in cuttings are conducive to adventitious root formation has been controversial for years (Veierskov et al., 1982). Although this has been documented with many species, TNC : N ratios are not always reliable indicators of rooting ability (Veierskov et al., 1982). In this study, TNC : N ratio tended to increase over time and was highest in cuttings taken from stock plants receiving N at 10 mg·L^{-1} (Fig. 3G). However, no correlation was found between TNC : N ratio and rooting.

Where differences existed between the two composite families, family GW contained greater quantities of myo-inositol, glucose, fructose, sucrose, total soluble carbohydrates (TSC), and TNC than family BR (Table 4). Sucrose exhibited a significant week × family interaction as GW contained equal or greater levels through week 9, but BR contained higher levels thereafter. There were no differences between families in starch content or TNC : N ratio. The increase in myo-inositol was greater in cuttings of the slower growing, but better-rooting family GW (Tables 1 and 3). This agrees with findings of Tschaplinski and Blake (1989), who reported that concentrations of glucose and myo-inositol declined more in faster-growing hybrids of poplar (*Populus* L.) than in slower-growing hybrids. Myo-inositol has been identified as essential for growth and differentiation in tissue cultures (Verma and Dougall, 1979) and an essential cofactor for cytokinin activity, which stimulates tissue development (Leopold and Kriedemann, 1975). Furthermore, polyols such as myo-inositol can alter the osmotic potential of cells at the base of the cutting and may promote the uptake of water (Loach, 1988).

Conclusion. Initial mineral nutrient and carbohydrate levels (with the exception of starch) were generally higher in cuttings taken from stock plants receiving the higher applied-N rates, and tended to remain higher throughout the 12-week rooting period. Although an ideal CHO : N ratio was not found, rooting can be improved by manipulating stock plant fertilization practices. Cuttings removed

from stock plants receiving the lowest N-fertilization rate rooted poorly, but had higher survival rates. In addition, genetic variation may play an important role, as families may respond differently to varying applied-N levels. Manipulating stock plant N nutrition influences adventitious rooting in loblolly pine, and probably in other difficult-to-root species as well. However, specific fertility regimes will need to be determined for each species and even for families or clones within species.

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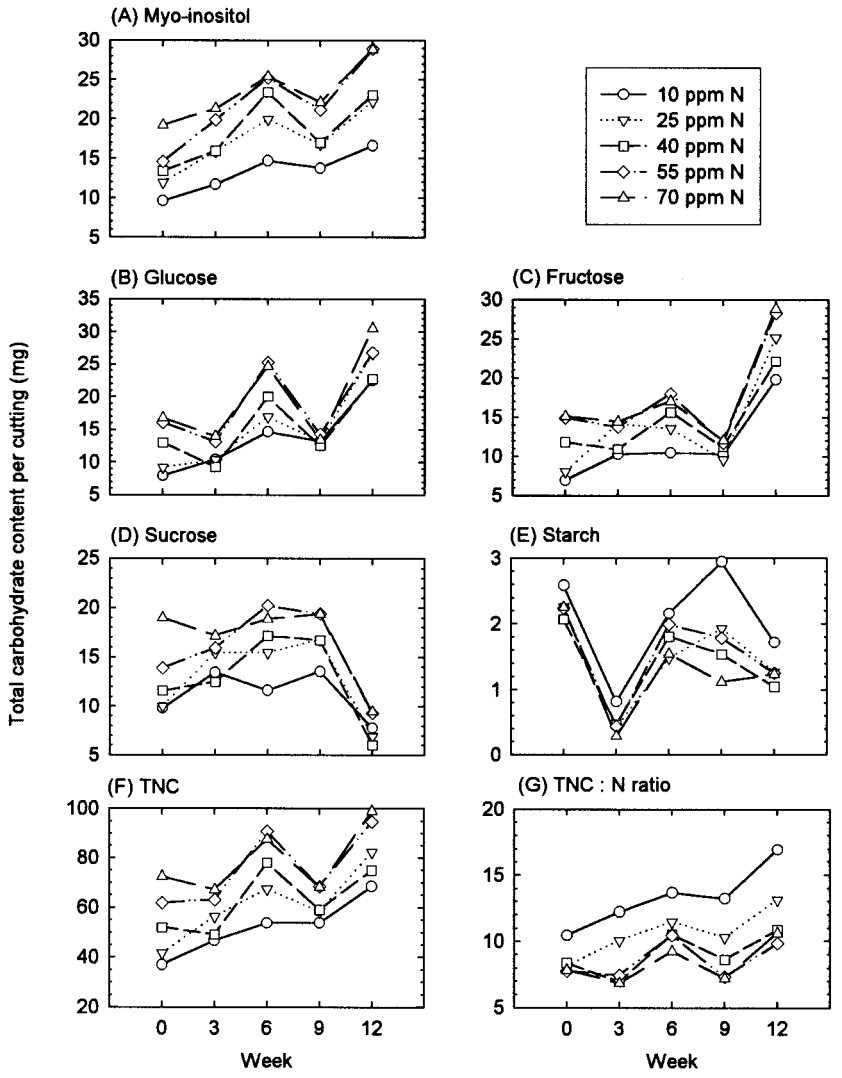

Fig. 3. Effect of stock plant nitrogen fertilization on carbohydrate content of stem cuttings of loblolly pine taken from hedged stock plants during a 12-week rooting period under intermittent mist. (**A**) myo-inositol; (**B**) glucose; (**C**) fructose; (**D**) sucrose; (**E**) starch; (**F**) total nonstructural carbohydrates (TNC); and (**G**) TNC : N ratio. Each symbol is based on eight means. Data are averaged over families.

Table 4. Carbohydrate content (mg) in stem cuttings of loblolly pine as influenced by family and time. Data averaged over stock plant N treatment. (Means based on 20 composite samples.)

		Week				
Carbohydrate	Family	0	3	6	9	12
Myo-inositol	BR	13.7 a[z]	15.5 b	21.0 a	16.6 b	22.3 b
	GW	13.8 a	18.4 a	22.5 a	19.7 a	25.6 a
Glucose	BR	10.8 b	10.5 b	18.3 a	11.8 b	22.4 b
	GW	14.5 a	12.4 a	22.4 a	15.0 a	29.4 a
Fructose	BR	9.8 b	10.2 b	12.8 b	9.00 b	21.7 b
	GW	13.0 a	15.2 a	17.1 a	13.0 a	28.1 a
Sucrose	BR	12.3 a	14.1 b	16.0 a	15.7 b	8.5 a
	GW	13.4 a	15.7 a	17.3 a	18.6 a	7.3 b
Starch	BR	2.3 a	0.5 a	1.9 a	1.7 a	1.3 a
	GW	2.2 a	0.4 a	1.7 a	2.0 a	1.3 a
TSC[y]	BR	46.6 b	50.3 b	68.2 b	53.1 b	74.9 b
	GW	54.7 a	61.8 a	79.3 a	66.3 a	90.4 a
TNC[x]	BR	48.9 b	50.8 b	70.1 b	54.8 b	76.2 b
	GW	57.0 a	62.2 a	81.0 a	68.3 a	91.7 a
TNC : N[w]	BR	8.8 a	8.4 a	11.1 a	9.0 a	11.9 a
	GW	8.2 a	9.0 a	11.1 a	9.6 a	12.7 a

[z]Mean separation between families within each week by LSD, $P \leq 0.05$.
[y]TSC = Total soluble carbohydrates.
[x]TNC = Total nonstructural carbohydrates.
[w]TNC : N = TNC to nitrogen ratio.

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