Adventitious Rooting of Stem Cuttings of Loblolly Pine as Influenced by Carbohydrate and Mineral Nutrient Content of Hedged Stock Plants

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Hedged stock plants of four full-sib families (B, G, R, and W) of loblolly pine (*Pinus taeda* L.) were fertilized daily with a complete nutrient solution containing either 10, 25, 40, 55, or 70 ppm N. In May 1995 (spring softwood), July 1995 (summer softwood), and Jan. 1996 (winter hardwood) terminal stem cuttings were taken for tissue analysis and rooting studies. Spring cuttings rooted in the highest percentages (59.5%), followed by winter (40.5%), and summer (34.7%). Maximum rooting for spring (70.0%), summer (48.6%), and winter (55.6%) occurred with cuttings taken from hedges that received 55 ppm N. Winter values of total nonstructural carbohydrates (TNC) were twice levels present in spring or summer (32.8% vs. 17.1% and 16.3%), but levels remained relatively constant with increasing applied N. In contrast, average N concentrations were lower in winter (1.29% vs. 1.79% and 1.69%) and increased linearly with increasing applied N levels. Genetic differences among families were evident as families B, G, and W exhibited a quadratic response with maximum rooting of 61.1% at 70 ppm applied N, 62.4% at 55 ppm N, and 63.0% at 40 ppm N, respectively. The TNC : N ratio was not correlated with rooting and an optimal TNC : N ratio for rooting success was not found. However, optimal rooting occurred at concentrations ranging from 1.8% to 2.0% N for spring and summer softwood cuttings and at approximately 1.5% N for winter hardwood cuttings. Also, low tissue B concentrations, which were not detrimental for plant growth, may have severely inhibited adventitious root formation.

INTRODUCTION

When focusing on the stock plant in relation to adventitious rooting of stem cuttings, one must consider the effects of both the environment and genetics on the physiological processes within the stock plant, which in turn influence subsequent rooting. Two measures of physiological status which are influenced by stock plant environmental history are carbohydrate content and nitrogen content (Andersen, 1986; Moe and Andersen, 1988). Changes in the relative amounts of either within cuttings influence rooting (Haissig, 1986).

Total nonstructrual carbohydrates (TNC) influence rooting by providing energy reserves and carbon skeletons to support root initiation and growth (Haissig, 1986; Veierskov, 1988). On the other hand, slight deficiencies of N (not stress) within the stock plant generally promote root formation in cuttings, presumably due to restricted metabolism of stock plant carbohydrates (Moe and Andersen, 1988). When photosynthetically fixed CO_2 enters the carbohydrate pools, it is normally available to be metabolized further. However, if N is deficient, carbon cannot be metabolized since most organic compounds contain N. In this situation, surplus carbohydrates would then be available to support root formation in stem cuttings (Veierskov, 1988). Thus, the aforementioned serves as a hypothesis that low N status relative to available carbohydrates (high TNC : N ratio) results in a tendency for stored carbohydrates and current photosynthate to be directed into adventitious root formation.

In addition to environmental effects, correlations between TNC : N ratios and rooting have been shown to have a genetic component. Hyun and Hong (1968) studied the seasonal variation in TNC : N ratios of both easy- and difficult-to-root clones of pitch pine (*Pinus rigida* Mill) and reported that clones which rooted in high percentages had higher TNC : N ratios than clones which were difficult-to-root. Although precise endogenous and exogenous relations between N and adventitious rooting have not been established, carbohydrate to N ratios can be manipulated by varying N fertilization provided to the stock plants. Henry et al. (1992a and 1992b) reported optimal growth of eastern redcedar (*Juniperus virginiana* L.) when stock plants were fertilized weekly with 180 ppm N. However, optimal rooting occurred at only 20 ppm N. This work also demonstrated that external N availability may have influenced rooting via its effects on uptake and utilization of other mineral nutrients. Therefore, the objective of this research was to determine whether selected levels of applied N supplied to hedged stock plants of loblolly pine (*Pinus taeda*) influence adventitious rooting with respect to the carbohydrate and N content of the cuttings.

MATERIALS AND METHODS

Hedged stock plants with varying carbohydrate and N status were established by growing plants outdoors on a gravel container pad at a range of applied N levels. The experimental design on the container pad was a randomized complete block design with four blocks each containing four full-sib families (controlled pollinations where both parents are known) and six N treatments (including an Osmocote control) arranged in a complete factorial, and with a four tree row plot within each block-family-N combination. A group of four-trees represented an experimental unit. There were a total of 24 treatments and 384 trees.

Trees for the five N treatments were grown in a medium of perlite and sand (6 : 4, v/v), while controls were grown in a medium of peat, coarse vermiculite, and perlite (2 : 2 : 1, by volume) amended with 2.1, 0.24, and 1.0 kg m^{-3} (3.6, 0.4, and 1.7 lb yd^{-3}) Osmocote $18N\text{-}6P_2O_5\text{-}12K_2O$, Micromax, and dolomitic lime, respectively. The six N treatments consisted of the peat culture control and five levels of N (10, 25, 40, 55, and 70 ppm N) supplied at optimum levels. The four families (designated B, G, R, and W) were included to compare genetic effects. From previous work it was determined that families B and R were poor rooting families (<10%) and families G and W were good rooters (>50%).

During May 1995 (spring softwood), July 1995 (summer softwood), and January 1996 (hardwood), 9-cm-long (3.5-in) terminal stem cuttings were taken from the hedged stock plants for tissue analysis and rooting experiments. These dates also coincided with rehedging of stock plants to maintain juvenility (Hackett, 1988). Tissue collected for carbohydrate and mineral nutrient analysis were lyophilized, ground to pass a 20-mesh (1.3-mm openings) screen, and extracted four times in 80%

ethanol. The decanted supernatant used to determine soluble carbohydrates was evaporated, resolubulized in deionized distilled H_2O, and centrifuged through microfilter columns packed with anion and cation exchange resins and polyvinylpolypyrrolidone (PVPP) to remove phenolic compounds. Samples were then analyzed for glucose, fructose, sucrose, raffinose, and the sugar alcohols, myoinisitol, and pinitol utilizing high performance liquid chromatography. The remaining insoluble pellet from the extraction process was utilized for enzymatic determination of starch. The samples were incubated with the enzymes amyloglucosidase, hexokinase, and glucose-6-phosphate dehydrogenase, and absorbance at 340 nm was measured on a spectrophotometer to determine starch content. In addition, tissue samples were analyzed with a CHN elemental analyzer to determine total C and N and by plasma emission spectrometer to determine P, K, Ca, S, Mg, Mn, Fe, Zn, B, and Cu. Cuttings utilized for rooting experiments were inserted into flats containing a medium of perlite and coarse vermiculite (1 : 1, v/v) and placed in a greenhouse under intermittent mist. They were not treated with auxin. After 12 weeks, cuttings were evaluated for percent rooting. A cutting having at least one root > 1 mm (0.04 in.) in length was considered rooted. Means were subjected to analysis of variance procedure and regression analysis (SAS Institute, 1990).

RESULTS AND DISCUSSION

There were significant differences among seasons, families, and N treatments, as well as a family × N interaction in regards to rooting percentages. When averaged over families and N treatments, significantly greater rooting percentages occurred for spring softwood cuttings (59.5%) than summer softwood (34.7%) or winter hardwood cuttings (40.5%). Maximum rooting for spring (70.0%), summer (48.6%), and winter (55.6%) occurred with cuttings taken from hedges that received 55 ppm N (Fig. 1A). Likewise, differences were evident in the percentage of total nonstructural carbohydrates (TNC) and percent N concentrations of the cutting tissue. Winter values of TNC were twice levels present in spring or summer (32.8% vs. 17.1% and 16.3%), but levels remained relatively constant with increasing applied N (Fig. 1B). In contrast, average N concentrations were lower in winter (1.29% vs. 1.79% and 1.69%) and increased linearly with increasing applied N levels (Fig. 1C).

When averaged over seasons, families B, G, and W exhibited a quadratic response with maximum rooting of 61.1% at 70 ppm applied N, 62.4% at 55 ppm N, and 63.0% at 40 ppm N, respectively (Fig. 2A). Overall, family R was the poorest rooting family. However, rooting increased linearly with higher applied N suggesting that additional N may have improved rooting further. Families G and W were the best rooting families at the lower applied N levels, whereas family B was extremely poor (8.3% at 10 ppm N). However, family B was the best rooting family at the highest applied N level (61.1% at 70 ppm N). These results emphasize that differences in rooting response are partially genetic, even within the same species.

Levels of applied N showed similar linear and quadratic responses for TNC (Fig. 2B). Families B and W contained the highest levels of TNC and were both good rooting families, whereas family R contained the lowest TNC levels and was the poorest rooting family. Family G was an exception. Although it was generally a good rooter, it contained low TNC levels, which suggests that it may be more efficient in metabolizing carbohydrates. Contrary, to our hypothesis, TNC : N ratio was not

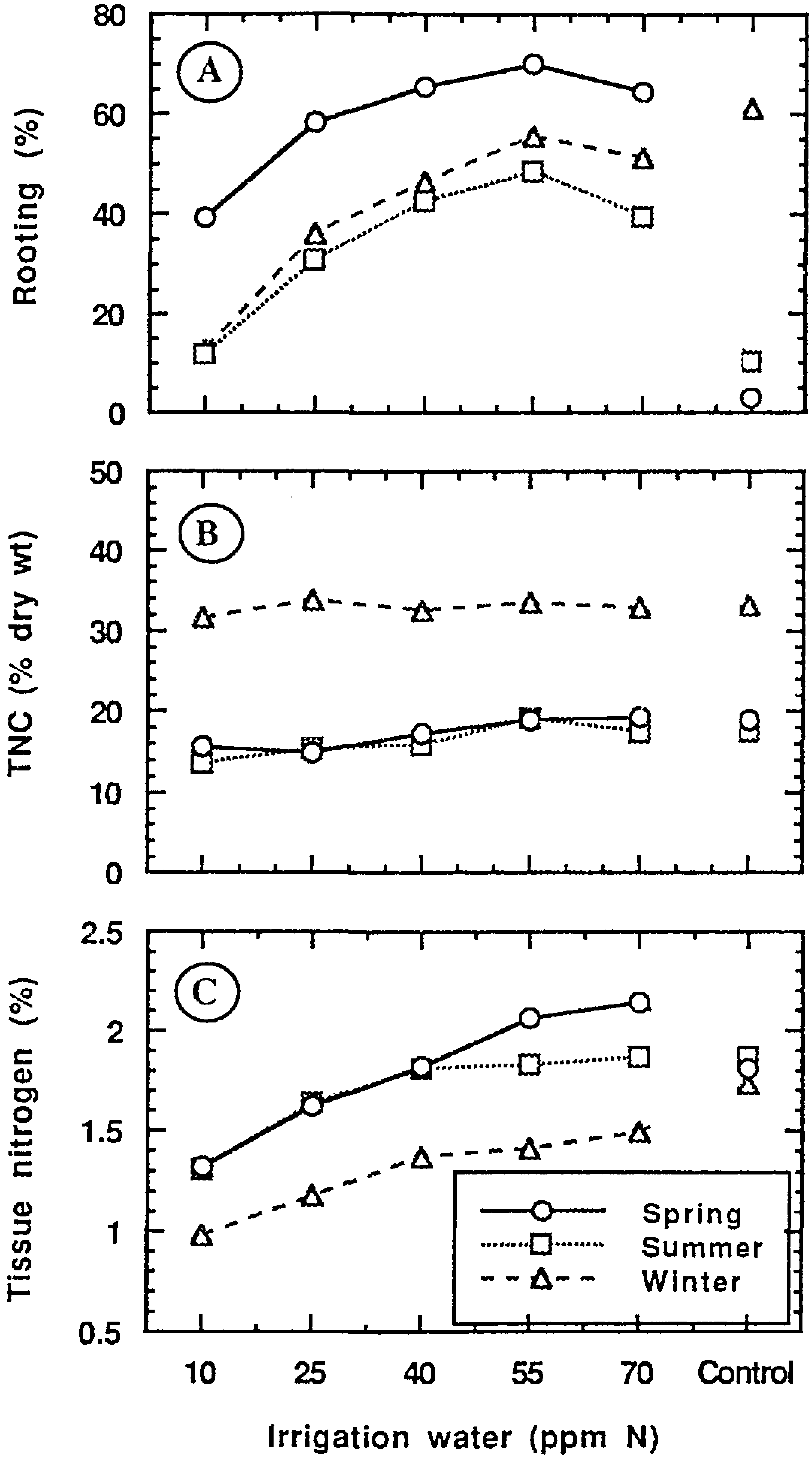

Figure 1. Effect of stock plant nitrogen fertilization on (A) rooting, (B) total nonstructural carbohydrates (TNC), and (C) tissue N concentrations of stem cuttings of loblolly pine taken from hedged stock plants. In (A), each symbol is based on 96 observations. In (B) and (C), each symbol is based on 16 observations. Data are averaged over families.

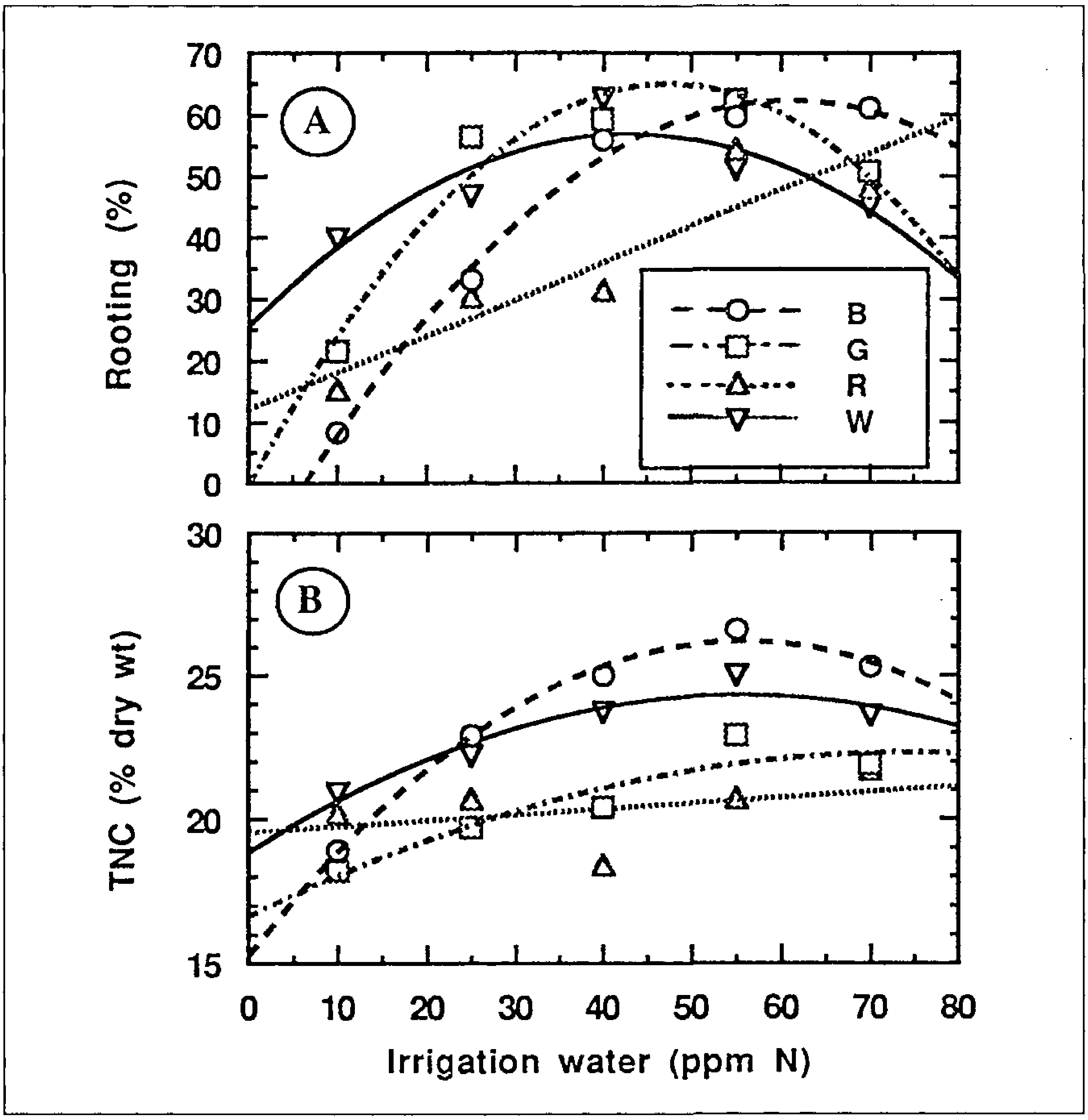

Figure. 2. Effect of stock plant nitrogen fertilization on (A) rooting and (B) total nonstructrual carbohydrate concentration (TNC) of stem cuttings of loblolly pine taken from four families (Fam. B, G, R, or W) of hedged stock plants. In (A), each symbol is based on 72 observations. In (B), each symbol is based on 12 observations. Data are averaged over seasons.

correlated with rooting and an optimal TNC : N ratio for rooting success was not found. As TNC for each family exhibited a quadratic or positive linear response to applied N, TNC : N ratio decreased with increases in applied N for all families (Fig. 3). At the lower applied N levels, tissue N concentrations were depressed (Fig.1C), which in turn inflated the TNC : N ratios. Even so, determining this ratio would be too time consuming and costly to be practical for a propagator. A more reasonable test would be tissue N concentration. For all treatments studied, a range of tissue N concentrations from 0.92% to 2.24% was observed. However, optimal rooting occurred at concentrations ranging from 1.8% to 2.0% N for spring and summer softwood cuttings and at approximately 1.5% N for winter hardwood cuttings.

Of the other mineral nutrients, B may have had the greatest impact on rooting. There were no significant differences among families or applied N levels, but a B

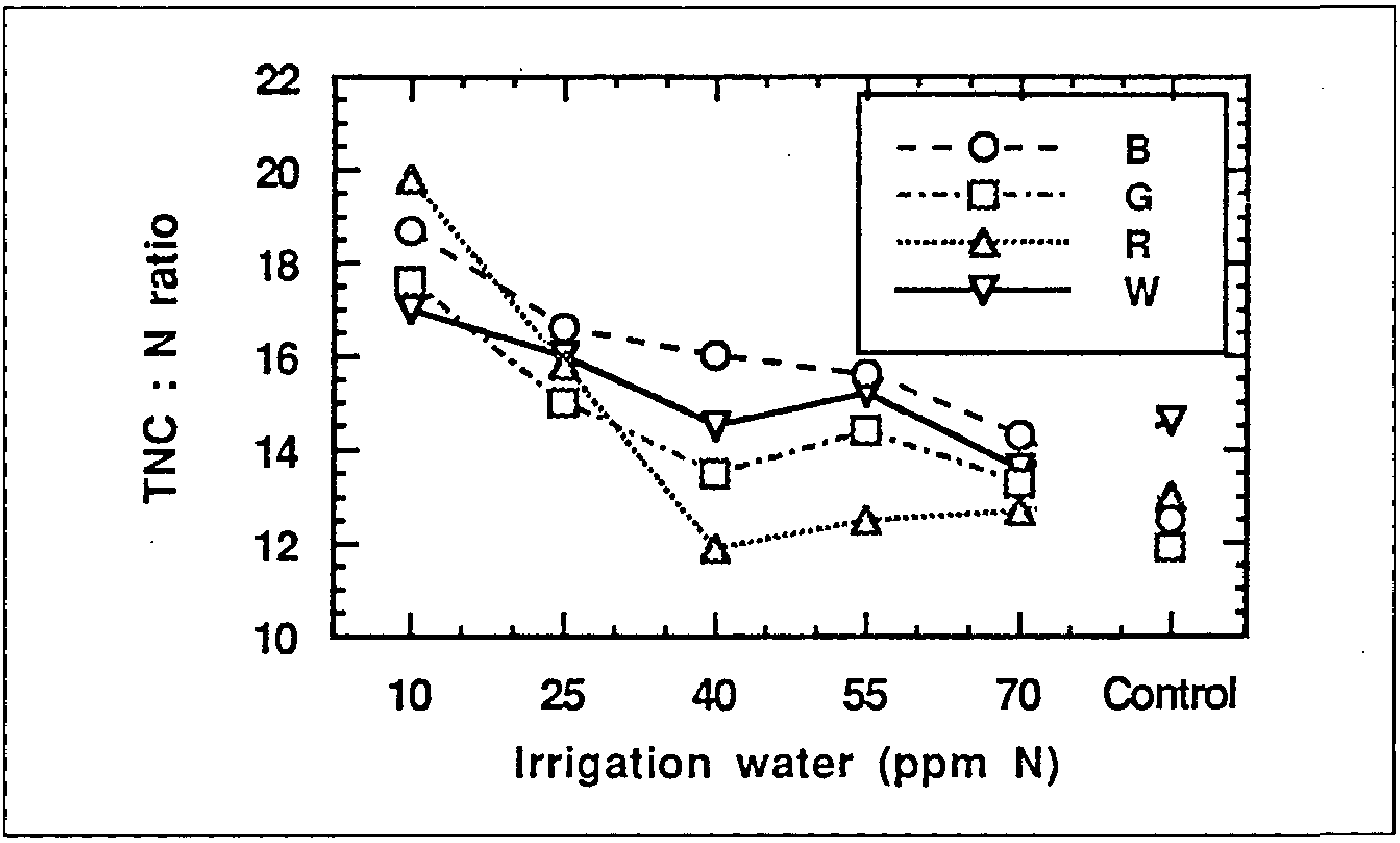

Figure. 3. Effect of stock plant nitrogen fertilization on total nonstructural carbohydrate : nitrogen (TNC : N) ratio of stem cuttings of loblolly pine taken from four families (Fam. B, G, R, or W) of hedged stock plants. Each symbol is based on 12 observations. Data are averaged over seasons.

deficiency in the spring (7.0 ppm) and summer (8.7 ppm) control treatments compared to the other 5 N treatments (21.7 ppm B), could have reduced rooting dramatically (Table 1). Rooting percentages for the controls were only 0.83% in spring and 10.4% during summer, but increased to 61.1% for winter cuttings when tissue levels were restored to 15.9 ppm B. 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 (data not presented). Even though 7.0 ppm B is adequate for plant growth, it appears that higher amounts may be required for adventitious rooting. Thus, further stimulation of rooting also may be possible by manipulating stock plant B concentrations.

Table 1. Internal boron concentration (ppm) in tissue of stem cuttings.

Applied N (ppm)	Spring	Summer	Winter
10	23.5	21.1	18.5
25	21.5	23.2	16.4
40	21.6	23.0	15.9
55	21.0	21.6	14.7
70	22.0	19.7	14.8
Control	7.0	8.7	15.9

In conclusion, the time of year in which woody stem cuttings are taken from stock plants (actually the growth stage), mineral nutrient status of stock plants, and genetic variation all have a major influence on adventitious rooting of loblolly pine. Spring softwood cuttings rooted in the highest percentages, followed by winter hardwood, and summer softwood cuttings. Also, genetics plays an important role in adventitious rooting as not all families responded the same to varying applied N levels. Manipulating stock plant 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 a species.

LITERATURE CITED

Andersen, A.S. 1986. Stock plant conditions, p. 223-255. In: M.B. Jackson (ed.). New root formation in plants and cutting. Martinus Nijhoff Publishers, Dordrecht, Netherlands.

Hackett, W.P. 1988. Donor plant maturation and adventitious root formation, p. 11-60. In: T.D. Davis, B.E. Haissig, and N. Sanhla (eds.). Adventitious root formation in cuttings. Dioscorides Press, Portland, Ore.

Haissig, B.E. 1986. Metabolic processess in adventitious rooting of cuttings, p. 141-190. In: M.B. Jackson (ed.). New Root formation in plants and cuttings. Martinus Nijhoff Publishers, Dordrecht, Netherlands.

Henry, P.H., F.A. Blazich, and **L.E. Hinesley**. 1992a. Nitrogen nutrition of containerized eastern redcedar. II. Influence of stock plant fertilty on adventitious rooting of stem cuttings. J. Amer. Soc. Hort. Sci. 117:568-570.

Henry, P.H., F.A. Blazich, L.E. Hinesley, and **R.D. Wright.** 1992b. Nitrogen nutrition of containerized eastern redcedar. I. Growth, mineral nutrient concentrations, and carbohydrate status. J. Amer. Soc. Hort. Sci. 117:563-567.

Hyun, S.K. and **S.O. Hong**. 1968. Fundamental mechanism of root formation in the cuttings of forest trees. Inst. For. Genet. Suwon, Korea, Res. Feg. 6:1-52.

Moe, R. and **A.S. Andersen.** 1988. Stock plant environment and subsequent adventitious rooting, p. 214-234. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). Adventitious root formation in cuttings. Dioscorides Press, Portland, Ore.

SAS Institute. 1990. SAS/STAT user's guide. Vol. 2. SAS Institute, Inc., Cary, N.C.

Veierskov, B. 1988. Relations between carbohydrates and adventitious root formation, p. 70-78. In: T.D. Davis, B.E. Haissig, and N. Sanhla (eds.). Adventitious Root Formation in Cuttings. Dioscorides Press, Portland, Oregon.