ROLE OF SHOOT GROWTH AND AMMONIUM NITRATE FERTILIZER ON ENDOGENOUS CARBOHYDRATE, AMMONIUM, AND NITRATE LEVELS OF NEWLY-ROOTED ACER PALMATUM CUTTINGS

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Abstract. Ammonium nitrate (NH_4NO_3) fertilization and shoot growth of *Acer palmatum* 'Bloodgood' were studied to determine their effects on carbohydrates and endogenous ammonium (NH_4) and nitrate (NO_3) levels. Generally, an inverse relationship existed between tissue NH_4 and NO_3 levels and soluble sugars (fructose, glucose, and sucrose). Exceedingly high NH_4 and concomitantly low soluble sugar amounts were apparent, especially in plants lacking new shoot growth and given N after rooting. Increasing the N rate magnified these trends. Plants with new shoot growth and given N maintained higher soluble sugar levels and lower NH_4 levels than plants without growth. These amounts remained relatively unchanged as N rate increased. Nitrogen had no effect on starch levels. These results suggest that plants without shoot growth have low tolerance to NH_4NO_3 fertilization, and are subject to NH_4 toxicity brought about by a decrease in available carbohydrates. Plants with new shoot growth were able to assimilate large amounts of NH_4 without harmful effect, while at the same time increasing shoot growth.

REVIEW OF LITERATURE

The low overwinter survival of rooted cuttings of certain deciduous woody plants such as *Acer, Cornus, Hamamelis, Magnolia, Prunus, Rhododendron,* and *Viburnum* is a significant problem. Cuttings will root and appear alive and dormant in the autumn, but either die the first winter or after budbreak in the spring (4,5,6,7,9,12,13).

Propagation time, nitrogen, and shoot growth were recently found to affect overwinter survival of newly-rooted stem tip cuttings of *Acer palmatum* 'Bloodgood' and *Cornus florida* var. *rubra* (9,20,21). Propagation of *A. palmatum* and *C. florida* in May and June, respectively, resulted in a greater number of plants with shoot growth after rooting compared to those propagated later. May propagation of *A. palmatum* resulted in reduced overwinter survival while propagation date did not significantly affect overwintering of *C. florida* when N was excluded, regardless of shoot growth. Plants with shoot growth receiving N had higher survival, but less than those with shoot growth and not given N. Poorest overwintering occurred on both species given N that failed to grow prior to winter storage.

The objective of this research was to determine the relationship of shoot growth and NH_4NO_3 fertilizer on endogenous levels of NH_4 and NO_3 nitrogen, and soluble carbohydrates.

MATERIALS AND METHODS

Primary shoot tip cuttings of *A. palmatum* 'Bloodgood' were taken at Angelica Nursery Inc., Chesterville, Maryland on 22 May, 1985. Cuttings were momentarily submerged in water, wrapped in moist burlap and plastic and placed in wooden flats in the shade. Upon return to the University of Maryland, they were resubmerged in water, again wrapped in burlap and plastic and stored at 4°C overnight.

Cuttings were recut to 20 cm and basal leaves were removed so that 4 or 5 fully expanded leaves remained. To aid rooting, four 2.5 cm vertical basal wounds through the cambium were made with a razor blade and the cuttings were dipped in water and treated with a commercial talc powder preparation of indole-3-butyric acid at 20,000 mg·g^{-1} (Hormo-Root 2; Hortus Products Inc., Newfoundland, New Jersey). Cuttings were placed in a moistened rooting medium composed of coarse perlite and sphagnum peat (3:2, v/v). To reduce transplant shock, cuttings were placed in styrofoam "Speedling" (Speedling Manufacturing, Inc., Sun City, Florida) trays with individual 3.75 × 3.75 × 12.5 cm compartments, and rooted under intermittent mist in the greenhouse.

After about 5 weeks, rooted cuttings (roots about 2.5 cm or longer) were transplanted into 10.2 cm square plastic pots (0.5 liter volume) with sphagnum peat and coarse perlite medium (3:2, v/v) amended with 7.5 g fritted trace elements #503, 525.0 g dolomitic limestone, 88.5 g $0N-44P_2O_5-0K_2O$, and 77.9 g $0N-0P_2O_5-50K_2O$ per 50 liters medium. The medium pH was 6.3. Potted plants were acclimated under decreasing levels of mist for 2 weeks.

After transplanting and acclimation, plants were placed in the greenhouse under an 18 hr photoperiod with day continuation (1600–0200HR) from 100 W incandescent bulbs placed 2 m apart and 1.1 m above the plants (9 μmols s^{-1}m^{-2}). Nitrogen treatments were applied as NH_4NO_3 once weekly at 0, 100, 200, and 400 mg·liter^{-1}. Pots were fertilized to runoff with ca 150 ml fertilizer solution. Water was provided to all plants in equal amounts between fertilizer applications.

Plants were arranged in a split-plot design with 2 blocks. Nitrogen was the whole plot. Within each whole plot, randomly selected plants had a terminal leaf and petiole removed (10 July, 1985) to expose the subtending bud and promote apical growth (9). This determined the subplot grouping: growth vs. no growth.

Plants were harvested 25 October, 1985 and roots and stems with and without shoot growth after rooting were analyzed for NH_4 and NO_3 nitrogen, and for sucrose, glucose, fructose, and starch. Detailed description of analysis procedures are described elsewhere (17).

RESULTS

Ammonium in roots and stems was influenced by a significant N × growth interaction (Fig. 1) (17). Plants with growth which received N had NH_4 levels slightly higher than treatments without N. Increases in N rate on plants which grew had no significant effect on root or stem NH_4 content. If *A. palmatum* 'Bloodgood' failed to grow after rooting and N was not provided, root and stem NH_4 levels were low and similar to those which grew and did not receive N. Increasing N rate to plants without growth resulted in sharp increases of NH_4 in root and stem tissues. Highest NH_4 concentrations were found in stems.

Nitrate levels in roots and stems were affected by a significant N × growth interaction (Fig. 1) (17). As N rate increased, root NO_3 increased further in plants which grew than those without growth. Additional stem NO_3 was found in plants without shoot growth as N rate increased, compared to those with growth. Highest NO_3 concentrations were found in roots.

Carbohydrates of roots and stems were influenced by N rate
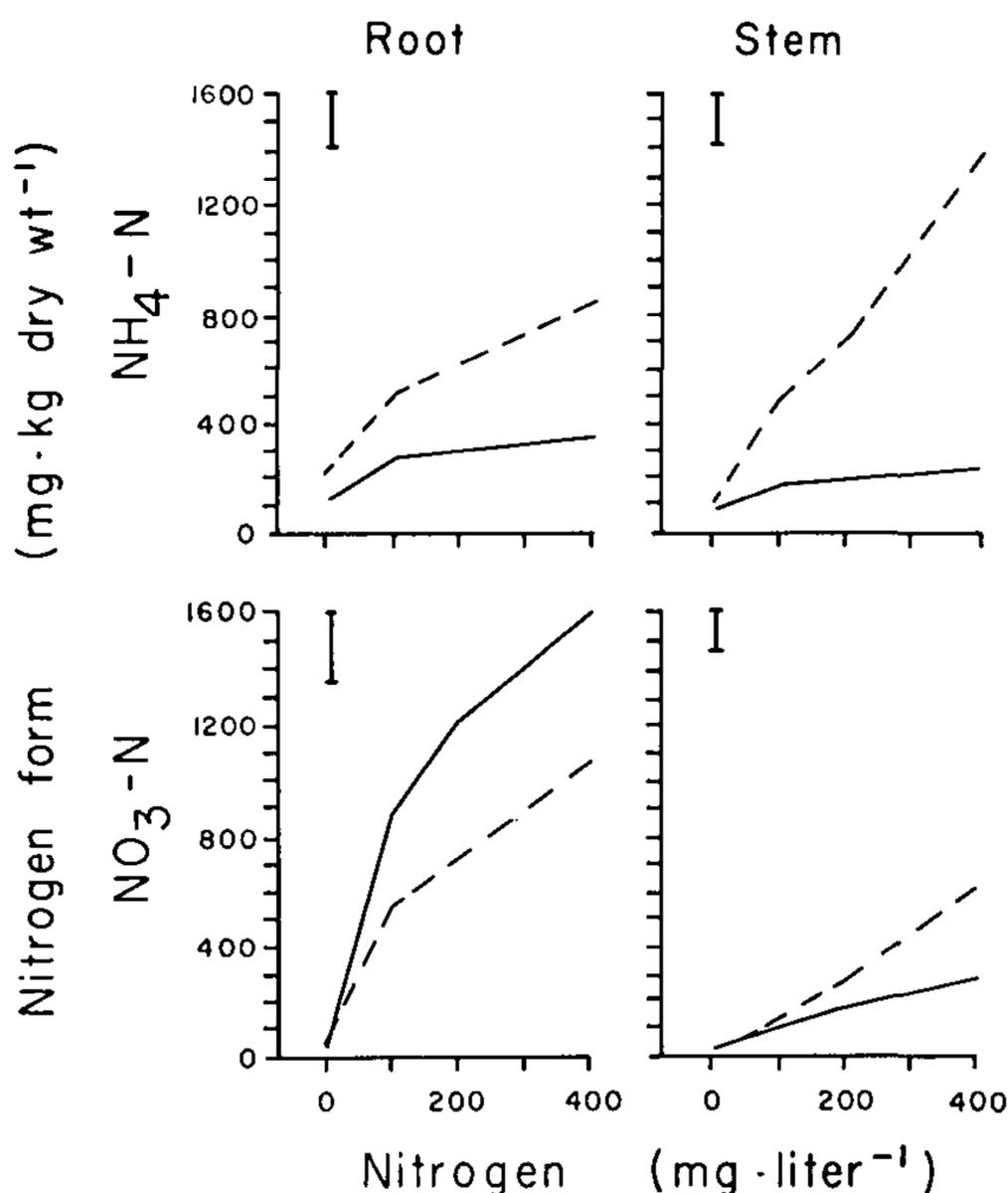

Figure 1. Ammonium and nitrate accumulation in roots and stems of rooted *Acer palmatum* 'Bloodgood' stem tip cuttings in response to nitrogen rate and plant growth. Lines represent plants with (———) and without (----) shoot growth after rooting. Vertical bars represent Fisher's LSD.

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and shoot growth after propagation [17]. Root fructose and glucose content and stem glucose were affected significantly by N and growth, and stem fructose by a N $\times$ growth interaction. A N $\times$ growth interaction for root and stem sucrose was significant [17]. Plant starch levels were not affected by nitrogen, but were influenced by plant growth, as seen in stem tissues.

Fructose and glucose levels of roots and stems were highest when treatment excluded N (Fig. 2). These levels were reduced sharply by addition of 100 mg·liter $^{-1}$ N, and were depleted further (though more slowly) by higher N rates. Nitrogen had little effect on root and stem sucrose in plants which grew. Regardless of N, plants with shoot growth after rooting generally maintained higher sucrose levels than plants without growth that were given N. No clear trend in starch level was apparent, regardless of shoot growth or N rate.

DISCUSSION

These data suggest that endogenous NH_4 and NO_3 levels, and fructose, glucose, and sucrose levels were associated with NH_4NO_3 fertilization rate and shoot growth response after rooting of A. palmatum 'Bloodgood' stem cuttings. Generally, an inverse relationship existed between tissue NH_4 and NO_3 levels, and soluble sugars. Exceedingly high tissue NH_4 and concomitant low soluble sugar concentrations were apparent, especially on plants lacking new shoot growth and given NH_4NO_3 after rooting (Figs. 1 and 2). Increasing the N rate enhanced the magnitude of separation between these trends. Since NH_4 assimilation into amino acids has a carbohydrate requirement [1,14,16,18], NH_4NO_3 fertilization would be expected to deplete carbohydrate levels. Acer palmatum with shoot growth and given NH_4NO_3 maintained higher soluble sugar levels and lower NH_4 levels than plants without growth. Interestingly, on plants with growth, the levels of endogenous NH_4 and soluble sugars remained relatively unchanged as NH_4NO_3 was increased. This suggests that, as a result of shoot growth, soluble sugar levels were sufficient to offset the carbohydrate demands of NH_4 assimilation brought about by NH_4NO_3 fertilization. If plants produced no shoot growth after propagation, low soluble sugar levels may have prevented assimilation of all cellular NH_4 allowing toxic amounts to accumulate.

Any role of starch in NH_4 assimilation remains unclear. Increasing NH_4NO_3 rate had no significant effect on endogenous starch levels in roots, stems or new shoots, even after NH_4 had reached toxic levels. Perhaps A. palmatum maintains its starch reserves at a given level, and must assimilate NH_4 at the expense of other carbohydrates. The rooted cuttings may be unable to hydrolyze starch into glucose, and must rely solely on existing fruc-
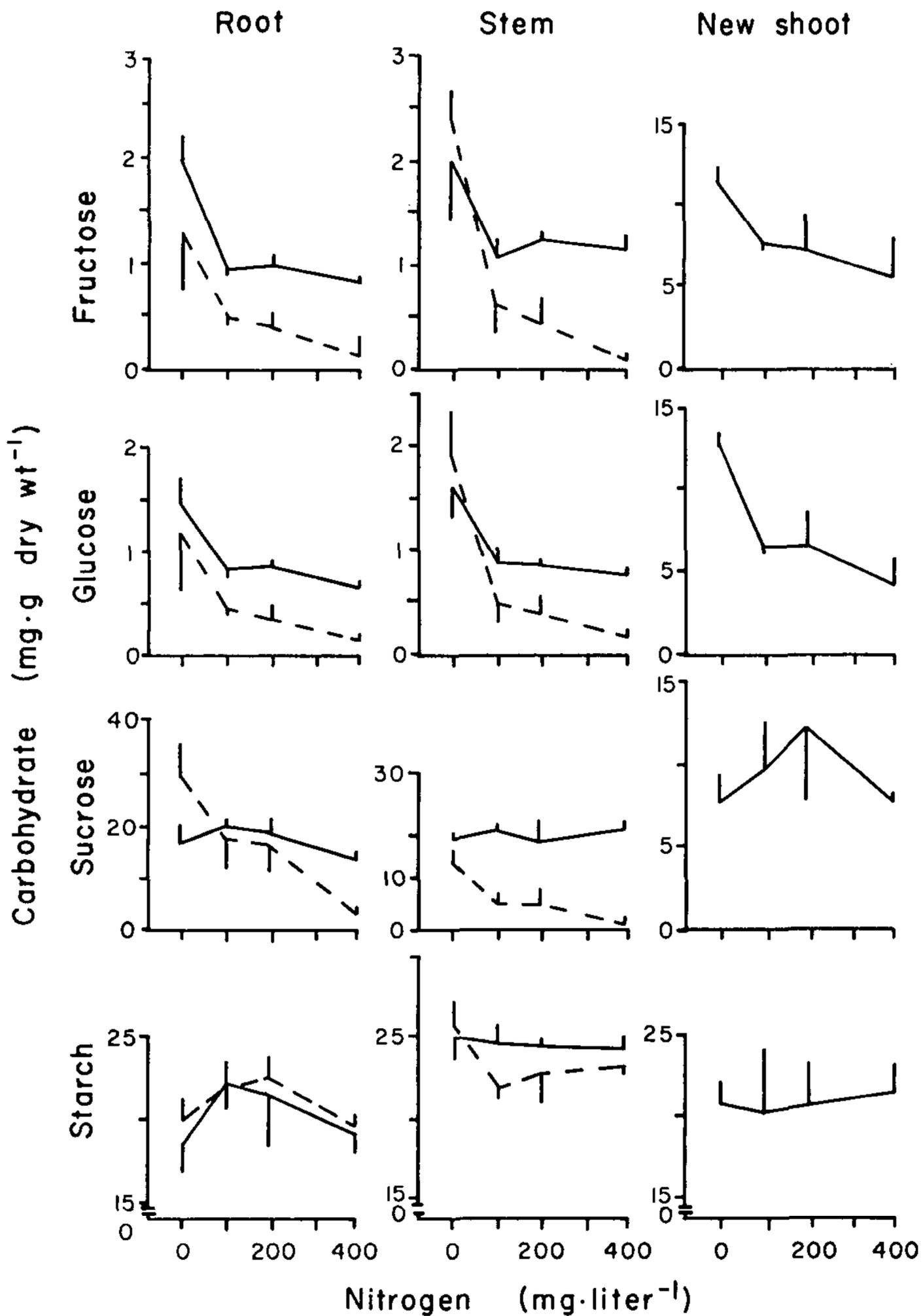
Figure 2. Influence of nitrogen rate and plant growth on carbohydrate levels in roots and stems of rooted *A. palmatum* 'Bloodgood' stem tip cuttings. Lines represent plants with (———) and without (----) shoot growth after rooting. Vertical bars represent standard errors.

tose, glucose, and sucrose as a carbon source for NH_4 assimilation. In contrast, in research with *A. rubrum* 'October Glory', starch levels increased in plants which grew after rooting (19). However, the role of nitrogen was not part of this study.

Once taken into the root, NH_4 is usually assimilated immediately into non-toxic glutamine (8). If inadequate carbohydrate supplies delay complete assimilation, however, NH_4 ions can accumulate in roots (18). This NH_4 may be transported to the stems and leaves, where it effects can be especially deleterious (2). Apparently this was the case in *A. palmatum*, where plants given NH_4NO_3 without shoot growth accumulated NH_4 in both roots and stems (Fig. 1). There were no increases in root or stem NH_4 in plants that grew. Increases in shoot NH_4 may be due to accelerated transpiration (and nutrient uptake) in plants with new growth. High transpiration has been associated previously with accumulation of shoot NH_4 sometimes in quantities sufficient to trigger NH_4, toxicity through uncoupled photophosphorylation (1,10,11).

Nitrate N, which is non-toxic, may be accumulated safely in cell vacuoles in relatively large quantities (1). Since NO_3 must be reduced before assimilation can occur, its usage requires more energy than NH_4 assimilation. Because of this, regulatory mechanisms within the plant limit NO_3 assimilation, periods of optimum efficiency (3). Nitrate reduction in roots occurs only when there is sufficient carbohydrates to produce the energy required for reduction and, at the same time, carbon skeletons for assimilation (15). The present data show that NO_3 is apparently not as mobile as NH_4 within *A. palmatum*, and accumulates in the roots after absorption (Figure 1). This finding may indicate the location of NO_3 reduction in the plant. Since NO_3 reductase is an adaptive enzyme, and is found only in the presence of NO_3, it probably exists in the highest quantities in the roots of *A. palmatum*. Plants with new shoot growth were found to accumulate larger quantities of NO_3 than plants lacking shoot growth, perhaps due to increased uptake of soil solution.

The results of this research indicate that plant growth after rooting of *A. palmatum* stem cuttings is essential to survival of plants receiving N. If new growth is initiated, plants may be fertilized with up to 200 mg·liter^{-1} N (as NH_4NO_3) without appreciable decrease in survival (17). If plants do not grow after rooting, NH_4NO_3 fertilization should be avoided, since lower survival is eminent.

LITERATURE CITED

1. Barker, A. V. and H. A. Mills. 1980. Ammonium and nitrate nutrition of horticultural crops, p. 395–423. In: J. Janick (ed.). Hort Rev. AVI, Westport, CT.

2. Barker, A. V., R. J. Volk, and W. A. Jackson. 1966. Root environment acidity as a regulatory factor in ammonium assimilation by the bean plant. *Plant Physiol.* 41:1193–1199.

3. Beavers, L. and R. H. Hageman. 1983. Uptake and reduction of nitrate: bacteria and higher plants, p. 351–369. In: A. Lauchli and R. L. Bieleski (eds.) Encyclopedia of Plant Physiology. Vol 15A. Springer-Verlag, New York.

4. Carthaigh D. M. 1983. Gibberellic acid breaks summer dormancy in rooted cuttings of flowering almond (Prunus triloba). Plant Prop. 29:12–14.

5. Donnely, J. R. and H. W. Yawney. 1972. Some factors associated with vegetatively propagating sugar maples by stem cuttings. Proc. Inter. Plant Prop. Soc. 22:413–430.

6. Fillmore, R. H. 1951. A general review of woody plant propagation. Proc. Inter. Plant Prop. Soc. 1:40–50.

7. Fordham, A. J. 1976. Propagation of some Hamamelidaceae (Witchhazel Family). Proc. Inter. Plant Prop. Soc. 26:296–300.

8. Givan, C. V. 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18:296–300.

9. Goodman, M. A. and D. P. Stimart. 1987. Factors regulating overwinter survival of newly propagated stem tip cuttings of Acer palmatum Thunb. 'Bloodgood' and Cornus florida L. var. rubra. HortScience. 22:1296–1298.

10. Goyal, S. S., O. A. Lorens, and R. C. Huffaker. 1982. Inhibitory effects of ammoniacal nitrogen on growth of radish plants. I. Characterization of toxic effects of NH_4 on growth and its alleviation by NO_3. Jour. Amer. Soc. Hort. Sci. 107:125–129.

11. Ikeda, M. and Y. Yamada. 1981. Dark CO_2 fixation in leaves of tomato plants grown with ammonium and nitrate as nitrogen sources. Plant and Soil 45:163–168.

12. Loach, K. and K. N. Whalley. 1975. Use of lights, carbon dioxide enrichment, and growth regulators in the overwintering of hardy ornamental nursery stock cuttings. Acta Hortic. 54:105–116.

13. McConnell, J. F. and D. E. Herman. 1980. The effect of gibberellic acid and benzyladenine in inducing bud break and overwintering of rooted softwood cuttings. Proc. Inter. Plant Prop. Soc. 30:398–404.

14. Michael, G., P. Martin, and I. Owassia. 1970. The uptake of ammonium and nitrate from labelled ammonium nitrate in relation to the carbohydrate supply of the roots, p. 22–29. In: E. A. Kirkby (ed.). Nitrogen nutrition of the plant. Waverly Press, Leeds, England.

15. Miflin, B. J. and P. J. Lea. 1980. Ammonia assimilation, P. 169–202. In: P. K. Stumpf and E. E. Conn (eds.). The biochemistry of plants. Acad. Press, New York.

16. Noggle, G. R. and G. J. Fritz. 1983. Introductory Plant Physiology. 2nd ed. Prentice-Hall Inc., Englewood Cliffs, NJ.

17. Patterson, S. F., D. P. Stimart, J. Meisinger, and K. Gross. 1988. Carbohydrate, ammonium, and nitrate levels of newly rooted Acer palmatum Thunb. 'Bloodgood' in relation to shoot growth and ammonium nitrate fertilizer. Jour. Amer. Soc. Hort. Sci. 113:(In review).

18. Reisenauer, H. M. 1978. Absorption and utilization of ammonium nitrogen by plants, p. 157–189. In: D. R. Nielsen and J. G. MacDonald (eds.). Nitrogen in the Environment. Vol. 2. Acad. Press, New York.

19. Smalley, T. J., M. A. Dirr, and G. C. Dull. 1987. Effect of extended photoperiod on budbreak, overwinter survival, and carbohydrate levels of Acer rubrum 'October Glory' rooted cuttings. Jour. Amer. Soc. Hort. Sci. 112:459–463.

20. Stimart, D. P. and M. A. Goodman. 1985. Overwinter survival of newly-propagated stem cuttings of certain deciduous woody plants. Comb. Proc. Inter. Plant Prop. Soc. 35:526–532.

21. Stimart, D. P., M. A. Goodman, and E. N. Ashworth. 1986. The relationship of shoot growth and nitrogen fertilization to cold hardiness of newly rooted Acer palmatum Thunb. 'Bloodgood' stem cuttings. Scientia Hortic. 20:341–347.

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