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Ammonium and Nitrate Accumulation in Containerized Southern Highbush Blueberry Plants

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Abstract. **Ammonium and NO_3^- uptake and partitioning were monitored in 'Sharpblue' southern highbush blueberry plants (*Vaccinium corymbosum* L. interspecific hybrid) using 10% ^{15}N-enriched N. Shoots and roots were harvested at 0, 6, 12, 24, and 48 hours after labeling. The rate of NH_4^+-N uptake was higher than that of NO_3^--N uptake, averaging 17.1 vs. 8.6 g N/g plant dry weight per hour during the 48-hour period for NH_4^+- and NO_3^--treated plants, respectively. At the end of the 48 hours, NH_4^+-N accumulation averaged 79 mg N/plant compared to 40 mg accumulated by the NO_3^--N-treated plants. Similarly, the translocation rate of NH_4^+-N to shoots was higher than translocation of NO_3^--N to shoots (7.7 vs. 1.9 g N/g shoot dry weight per hour, respectively) during the 48 hours. Shoot accumulation of NH_4^+-N averaged 40 mg N/plant at the end of 48 hours, while accumulation in shoots of NO_3^--N-treated plants averaged 10 mg N/plant. Short-term NO_3^- uptake and translocation to shoots appears to be limited relative to NH_4^+ uptake and translocation in southern highbush blueberry when plants are previously fertilized with NH_4NO_3.**

Blueberry plants, which are indigenous to acidic soils that are usually higher in NH_4^+ than NO_3^--N, produce healthy vegetative growth when fertilized with NH_4^+, NO_3^-, or both (Peterson et al., 1988; Takamizo and Sugiyama, 1991). In many studies, more shoot and root growth has occurred when plants are fertilized with NH_4^+ compared to NO_3^- (Cain, 1952; Townsend, 1970). The reason for occasionally decreased vegetative growth in NO_3^--fertilized blueberry plants is unknown.

Ammonium and NO_3^- are taken up by plant roots; however, the uptake rate often differs between the two N forms (Frith and Nichols, 1975; Grasmanis and Nicholas, 1971). Apple (*Malus domestica* Borkh.) trees previously fertilized with NH_4NO_3 absorbed more ^{15}N-labeled NH_4^+ than ^{15}N-labeled NO_3^- for the first 24 h after labeling (Grasmanis and Nicholas, 1971). After 24 h, ^{15}N-labeled NH_4^+ uptake rates decreased, and by 7 days after labeling, accumulation of ^{15}N from ^{15}N-labeled NO_3^- and ^{15}N-labeled NH_4^+ was equal. In jack pine (*Pinus banksiana* Lamb.) seedlings, more NH_4^+ than NO_3^- was absorbed during the 24 h following application, regardless of whether seedlings had been supplemented with NH_4^+ or NO_3^- for the previous 3 months (Lavoie et al., 1992).

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It is unclear if differences in N accumulation were due to direct effects on uptake of NH_4^+ vs. NO_3^- or to differences in vegetative growth induced by the different N forms. However, conifers are similar to blueberry in that growth appears to be enhanced by NH_4^+-N compared to NO_3^--N (Scheromm and Plassard, 1988).

The location of N assimilation often is correlated with short-term (1 h to 15 days) N partitioning patterns. In many plants, assimilation of NH_4^+ into amino acids occurs primarily in roots, soon after uptake (Lewis et al., 1986, 1987). Nitrate assimilation occurs primarily in the leaves of most herbaceous crops (Dirr et al., 1972; Pate, 1980; Smirnoff and Stewart, 1985) and in the roots of many woody perennials (Dirr et al., 1972; Pate, 1980; Smirnoff and Stewart, 1985; Smirnoff et al., 1984; Townsend, 1970; Yandow and Klein, 1986). In blueberry, NO_3^- reductase activity (NRA) has been detected in the leaves and roots (Dirr et al., 1972, 1973; Townsend, 1970). In citrus trees, which have NRA in leaf tissue (Bar-Akiva and Sternbaum, 1965), a greater portion of NO_3^--derived N vs. NH_4^+-derived N had been translocated from the roots to the shoots 48 h after ^{15}N labeling (Serna et al., 1992). This result suggests that root-to-shoot NO_3^- transfer may have preceded much of the NO_3^- reduction.

Although many studies have described effects of N form on blueberry growth, there is limited information on the uptake and partitioning patterns of NH_4^+-N and NO_3^--N in blueberries. Our study quantifies short-term accumulation of NH_4^+-derived and NO_3^--derived N in blueberries by using ^{15}N-labeled N. Our objective was to determine if differences in N accumulation and partitioning existed between the two N forms when uptake was not influenced by growth differences.

Materials and Methods

Plant material. Two-year-old 'Sharpblue' blueberry plants were grown from rooted cuttings in 2-liter pots containing a 1 peat : 1 pine bark mixture (v/v). Plants were grown in a field nursery and were fertilized once a month, from Mar. through Oct. 1989 with 30 kg 12N–4P–8K/ha containing 50% urea and 50% $(NH_4)_2SO_4$ as the N sources. The soluble fertilizer was applied through the overhead irrigation system.

Sand culture procedure. In Mar. 1990, plants were removed from the field nursery and roots were cleaned of soil before being planted in acid-washed, 20/30-mesh, silica sand. Plants were fertilized every other day with 1 liter of a modified Hoagland's solution consisting of (mmol): 5.0 N, 1.4 P, 1.0 K, 0.5 Ca, 1.0 Mg, 0.4 Na, 1.7 S, 1.0 Cl, 9.0×10^{-2} Fe, 4.5×10^{-2} B, 9.1×10^{-2} Mn, 1.1×10^{-2} Zn, 1.6×10^{-3} Cu, and 2.1×10^{-3} Mo. The Fe source was FeEDTA chelate. Also, 1 liter of deionized water was applied on alternate days. All plants received nutrient solutions containing NH_4NO_3 as the N form. The initial pH of the nutrient solution was 5.0. Effluent pH was monitored every other week by collecting the leachate into trays placed under the pots. During the first 8 weeks of growth, effluent pH decreased gradually. To avoid excessively acidic solutions, nutrient solution pH was increased to pH 6.5 after 8 weeks and maintained there for the duration of the experiment. At this solution pH, effluent pH stabilized at 3.5.

Plants were grown for 25 weeks from Mar. to Sept. 1990 in a greenhouse where the temperatures averaged 30C day/22C night and the maximum light level [photosynthetic photon flux (PPF)] during the afternoon was 1200 $\mu mol\cdot m^{-2}\cdot s^{-1}$.

Nitrogen accumulation. To determine the accumulation of N, plants were labeled with 105 mg of 10% ^{15}N enriched N (Isotec; Miamisburg, Ohio) on 29 Sept. 1990. Treatments were a factorial arrangement of two forms of ^{15}N-enriched N [$(NH_4)_2SO_4$ or $NaNO_3$] in combination with time of harvest after labeling (0, 6, 12, 24, or 48 h). Fifty-four plants were used, which provided six replications of nine treatments. The same six replicates of the 0 time period were used for both N forms. The experiment was a randomized complete-block design. The block effect was included due to the temperature gradient that occurred in the greenhouse. Plants were midway through the development of the second vegetative flush at the time of labeling. Nitrogen was dissolved in 0.5 liter deionized water and applied as a soil drench at 9:30 AM, 2.5 h after sunrise. No ^{15}N-labeled solution leached out of the pots at the time of labeling, and no additional watering was done during the 48 h. During ^{15}N uptake, air temperatures on both days ranged from 22 to 30C. Maximum PPF was 635 and 1200 $\mu mol\cdot m^{-2}\cdot s^{-1}$ the first and the second day, respectively. After 0, 6, 12, 24, and 48 h, plants were harvested, divided into shoots and roots, oven-dried, and ground with a Wiley mill (Arthur H. Thomas Co., Philadelphia) to

pass through a 40-mesh (0.417 mm) screen.

Tissue N was determined by combusting and oxidizing 3-mg subsamples and quantifying the evolved N_2 on a NA 1500 gas chromatograph (Carlo Erba; Strada Riboltana, Italy). The atom percentage of ^{15}N was determined by mass spectrophotometry (Vaccume Generators 602E, Winsford, England). Calculations used to determine ^{15}N uptake and partitioning were those previously described by Shearer and Kohl (1993).

Statistical analysis. Nitrogen accumulation data were tested by analysis of variance and regression analysis. Regression models were selected based on the highest r^2 values. The uptake rates of NH_4^+ and NO_3^- were derived indirectly from calculations based on the ^{15}N accumulation data, where the mean ^{15}N accumulated at time 1 was subtracted from the mean ^{15}N accumulated at time 2. This quantity was converted to uptake rates by dividing by the mean plant dry weight and the time interval between time 1 and time 2.

Results and Discussion

Nitrogen uptake. Accumulation of ^{15}N-labeled fertilizer N in the whole plant, roots, and shoots was significantly greater in the NH_4^+- than in the NO_3^--labeled plants during the 48 h after labeling (Fig. 1). By 48 h, $^{15}NH_4^+$-labeled plants had accumulated 79 mg of ^{15}N-labeled fertilizer N while $^{15}NO_3^-$-labeled plants had accumulated 40 mg. The uptake rate of NH_4^+ was greater than the uptake rate of NO_3^- during the first 24 h after ^{15}N-labeling. The uptake rate of both forms declined rapidly during the first 18 h after labeling, at which time, NO_3^- uptake rate averaged ≈6.0 μg N/gram plant dry weight per hour, while the NH_4^+ uptake rate was about two-fold higher (Fig. 2). From about 24 hours, uptake rates were similar for both N forms (5.0 μg N/g plant dry weight per hour). The decline in N uptake rates may be attributed to the decrease in available $^{15}NH_4^+$ and $^{15}NO_3^-$ because ^{15}N-labeling was done with a single application at time 0, rather than a constant feed throughout the 48 h. The highest rates of NO_3^- uptake in our study were lower than rates for pine (Scheromm and Plassard, 1988), which in turn were significantly lower than rates for herbaceous plants (Pace et al., 1990; Rao and Rains, 1976). Conversely, the NH_4^+ uptake rates in our study were higher than those observed in many herbaceous plants (Causin and Barneix, 1993; Wang et al., 1993), although NH_4^+ uptake rates in N-depleted blueberry plants are reportedly much higher than those measured in our study (Sugiyama and Hirooka, 1993).

The faster uptake of NH_4^+ than of NO_3^- is similar to results observed in young western hemlock (*Tsuga heterophylla* Sarg.) seedlings previously fertilized with NH_4NO_3 (Knoepp et al., 1993) and Norway spruce (*Picea abies* Karst.) seedlings fertilized with either NH_4^+ or NO_3^- (Lumme, 1994). The decrease in $^{15}NO_3^-$ uptake relative to $^{15}NH_4^+$ uptake may be due to the inhibition of NO_3^- uptake by NH_4^+, which was applied to all plants as NH_4NO_3 before feeding with ^{15}N. Pilbeam and Kirkby (1992)

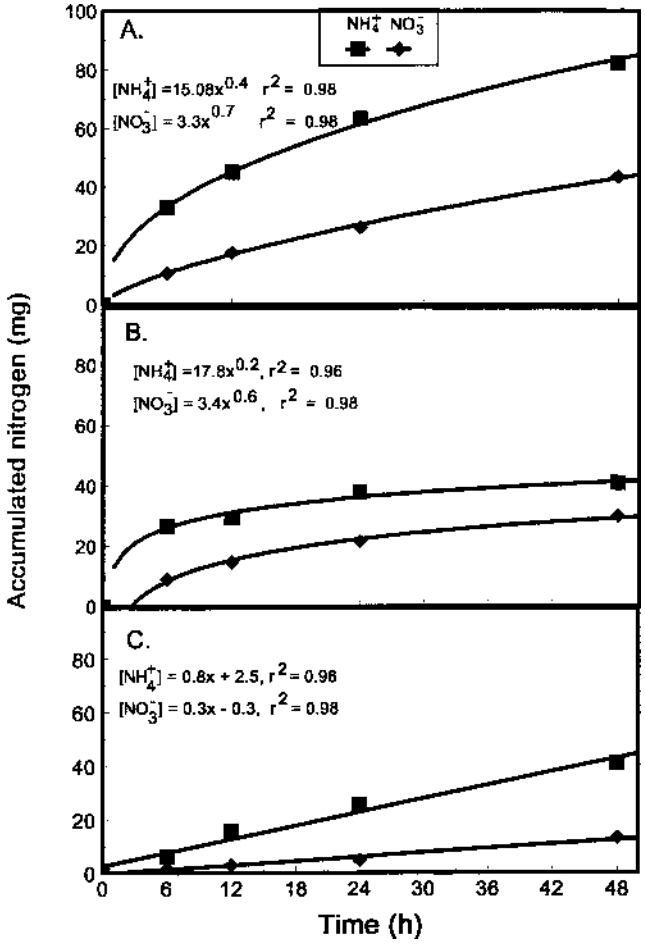

Fig. 1. Accumulation of ^{15}N-enriched fertilizer N in (**A**) the whole plant, (**B**) roots, and (**C**) shoots during 48 h following a single application of ^{15}N-labeled NH_4^+ or ^{15}N-labeled NO_3^-.

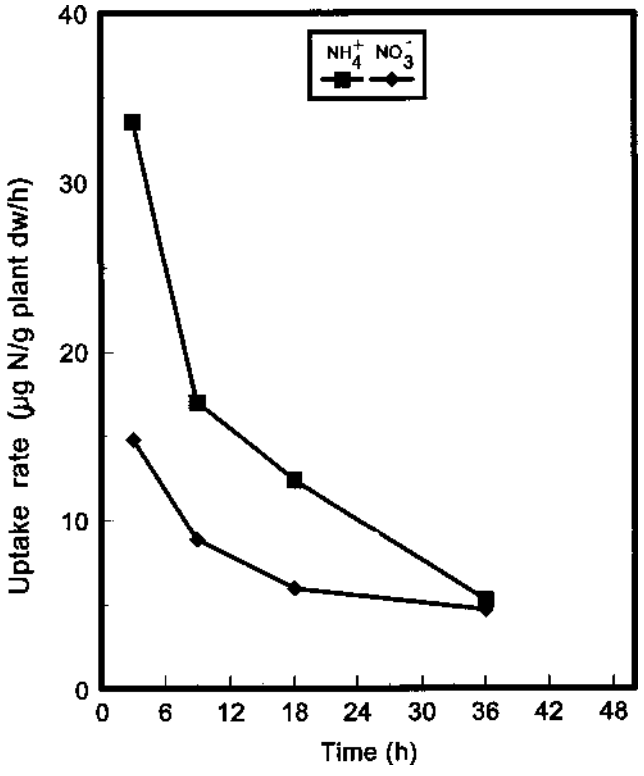

Fig. 2. Rate of ^{15}N-enriched fertilizer N uptake at intervals following a single application of ^{15}N-labeled NH_4^+ or ^{15}N-labeled NO_3^-. Data were calculated from Fig. 1A.

reported inhibition of NO_3^- uptake by NH_4^+ for numerous herbaceous plant species. Alternatively, the decrease in NO_3^- uptake may be associated with limited NRA in the roots, shoots, or both (Wallace, 1987). Previous work indicates that NRA in Ericaceous crops, such as blueberry, is low and predominates in the roots (Dirr et al., 1972; Smirnoff and Stewart, 1985; Smirnoff et al., 1984; Townsend, 1970).

In contrast, the higher rate of NH_4^+ uptake relative to NO_3^- uptake may be correlated with the enzymatic pathways used for NH_4^+ assimilation rather than the pathway required for NO_3^- reduction. Scots pine (*Pinus sylvestris* L.) seedlings exhibited increased glutamine synthetase (GS) activity when fertilized with NH_4^+ compared to NO_3^- (Seith et al., 1994). To our knowledge, the effects of NH_4^+ on GS and glutamate synthase (GOGAT) activity have not been examined in blueberry.

Nitrogen accumulation in shoots. Nitrogen accumulation in the shoots was linear for NH_4^+- and NO_3^--treated plants over the 48 h (Fig. 1C). Nitrogen translocation rate to shoots was more rapid for NH_4^+- than for NO_3^--derived N, averaging 11.9 and 4.56 µg N/g shoot dry weight per hour, respectively. Total accumulation of NH_4^+-derived N in shoots was 40 mg, while accumulation of NO_3^--derived N averaged 12 mg. The percentage of N taken up that was partitioned to shoots was similar for NH_4^+ and NO_3^--treated plants during the 0- to 6-h interval. However, between 6 and 48 h, the percentage increased significantly in the NH_4^+-fertilized plants compared to those fertilized with NO_3^- (Fig. 3).

Overall, the greater accumulation of NH_4^+-derived N vs. NO_3^--derived N in the shoots in our study agrees with results of long-term studies on blueberries where higher N concentrations were measured in shoots (Takamizo and Sugiyama, 1991) or roots and shoots (Rosen et al., 1990; Townsend, 1969) when plants were fertilized with NH_4^+ rather than NO_3^-. The limited translocation to shoots of NO_3^--derived N, compared to NH_4^+-derived N, may be correlated with low rates of NO_3^- reduction, which may limit NO_3^- uptake, assimilation, and eventual translocation to the shoots.

Based on these data, 'Sharpblue' blueberries are able to take up NH_4^+ and NO_3^-; however, NH_4^+ uptake is significantly greater than NO_3^- uptake. Similarly, translocation of NH_4^+-derived N is more extensive than N from NO_3^-, even when normalized for the amount of N taken up. Our study eliminated differences in plant growth as a significant factor contributing to differences in N uptake and translocation to shoots because plants grew uniformly. Therefore, the increase in NH_4^+ uptake and translocation compared to NO_3^- uptake and translocation in blueberry is a direct relationship, rather than an indirect result of growth differences. This difference in uptake may be due to either a limitation in NO_3^- uptake and translocation or an enhancement of NH_4^+ uptake and translocation. The former possibility may be related to insufficient activity or quantity of NO_3^- reductase in the roots and shoots, while the latter possibility may be related to increased assimilation rates for NH_4^+. Additional research is required to determine if either of these processes is correlated with decreased NO_3^- uptake or increased NH_4^+ translocation in blueberry.

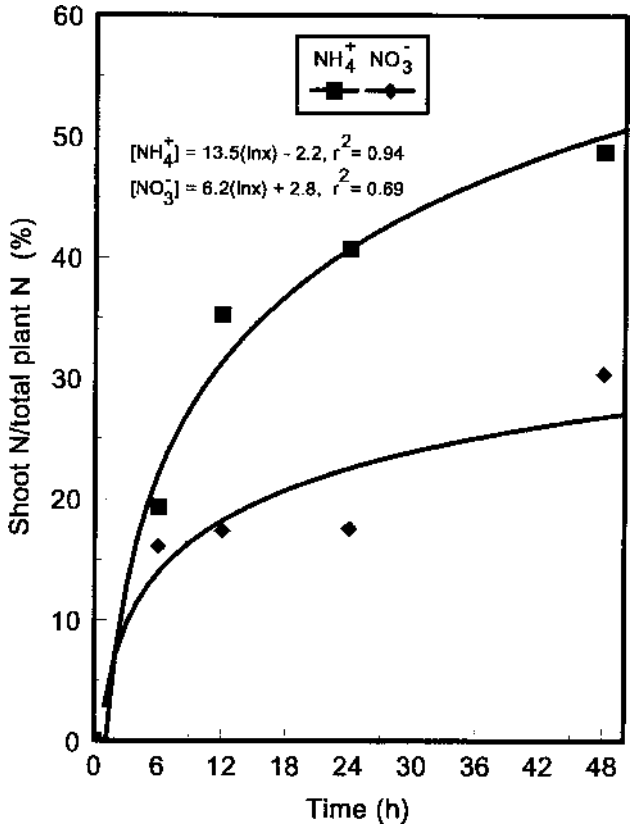

Fig. 3. Percentage of fertilizer N taken up that was accumulated in shoots during 48 h following a single application of ^{15}N-labeled NH_4^+ or ^{15}N-labeled NO_3^-.

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