

## **Progress Report**

**Title: Determining nitrogen-source preference in blueberry using a split-root system**

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### **Public Abstract**

Blueberry is a major fruit crop in Georgia and the southeastern US. Improved precision in its nutrient management is essential to ensure its sustained profitability in production. Blueberry plants are thought to display a preference for the ammonium form of nitrogen (N) in comparison to the nitrate form. The physiological basis for this preference remains unclear. In this study we used a split-root system to investigate N-source preference in southern highbush blueberry (Suziblue) at low and high levels of N-supply. Further, we compared N-source preference when the same plant was provided with both forms of N, one each to each half of the root system. At lower rates of N-supply, blueberry plants displayed a 3.6-fold higher preference for the ammonium form of N in terms of the N-uptake rate. At a higher N-supply rate, N-source preference for ammonium was still present but at a lower magnitude (< 2-fold). When compared within the same plant, a similar (4.6-fold) higher preference for N-uptake as ammonium was observed at lower N-supply levels. However, N-uptake rates were similar at higher N-supply. These data suggest that when N-availability is low, blueberry plants demonstrate N-source preference consistently for the ammonium form. This preference is substantially reduced or lost at higher N-availability. These differences may be due to the different transport systems operational at the different N-supply rates. The above information needs to be repeated and confirmed using other cultivars and rabbiteye blueberry genotypes to determine if these patterns are consistent across different types of blueberry.

## Introduction

Blueberry (*Vaccinium* species) is a major fruit crop in the southeastern United States. In Georgia, current cultivation of blueberry exceeds 30,000 acres with a farm gate value of over \$283 million, making it the most valuable fleshy fruit in the state (2016 Georgia Farm Gate Value Report). Rabbit-eye blueberry and southern highbush blueberry are the two main types of *Vaccinium* species grown in the state. Advances in increasing the efficiency of blueberry production of both these types are essential to sustain the profitability of blueberry production. An aspect of improved production efficiency is the ability to more precisely provide nutrients to the plant. This involves provision of the right nutrients, in a form that is most accessible, in the right amount based on developmental requirements, and in a site-specific manner. To achieve this, a clear understanding of the nutritional requirements of the plant is required. Blueberries have specific nutritional requirements. One such important nutritional requirement is their potential preference for the ammonium ( $\text{NH}_4^+$ ) form of inorganic N over the nitrate ( $\text{NO}_3^-$ ) form (Claussen and Lenz, 1999; Poonnachat and Darnell, 2004; Alt et al., 2017). The mechanisms behind this nutritional preference remain unclear. Also, it is not well understood whether all types of blueberry exhibit such preference, especially since the southeast has witnessed the emergence of newer rabbit-eye and southern highbush blueberry genotypes over the last decade. Thorough knowledge of such nutritional preferences especially in emerging cultivars is essential to precisely provide nutrients in the most efficient way in blueberry production. The goal of the proposed work is to characterize the N-source preference in several emerging rabbit-eye and southern highbush blueberry genotypes using a split-root system.

Nitrogen is a macronutrient that constitutes around 2% of the leaf dry weight in blueberries (Korcak, 1988). It is typically present in multiple forms in the soil but the major inorganic forms available for plant acquisition are  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Acquisition of  $\text{NO}_3^-$  from the soil into root cells is an energy-consuming process mediated by specific transporter proteins. Once acquired,  $\text{NO}_3^-$  can be stored, assimilated or translocated to other parts (shoots) of the plant. Assimilation of  $\text{NO}_3^-$  involves its reduction to nitrite and further to  $\text{NH}_4^+$ , catalyzed by nitrate reductase (NR) and nitrite reductase (NiR), respectively. Reduction of  $\text{NO}_3^-$  is an energy-intensive process requiring the availability of multiple reducing equivalents. Acquisition of  $\text{NH}_4^+$  from the soil into root cells is also mediated by transporter proteins. Typically,  $\text{NH}_4^+$  is assimilated at the site of acquisition through its addition to amino acids and subsequent transfer of amino groups among organic molecules. Ammonium generally does not accumulate in large quantities due to its potential toxicity (Britto et al., 2001).

Some plants display a preference for acquisition and utilization of one form of inorganic N over the other, a phenomenon termed as N-source preference (Britto and Kronzucker, 2013). Plants of the Ericaceae family, including blueberry, are thought to display a preference for the  $\text{NH}_4^+$  form of inorganic N. In blueberry, some reports

suggested a decline in plant growth when only  $\text{NO}_3^-$  was provided as the N source (Townsend, 1969; Claussen and Lenz, 1999). N-source preference may be associated with differences in the acquisition, translocation, or assimilation capacities for the form of interest. In blueberry, an overall lower capacity for  $\text{NO}_3^-$  reduction, especially within the shoots was suggested to result in lower uptake of this form of N (Poonnachit and Darnell, 2004). Previous research by the PI's group indicated that the shoot capacity for  $\text{NO}_3^-$  assimilation was inducible when  $\text{NO}_3^-$  was directly supplied to this tissue (Alt et al., 2017). Further, the rate of the N uptake as  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was similar in one each of the rabbiteye and southern highbush blueberries tested. These observations suggested potential limitations in  $\text{NO}_3^-$  loading and its translocation as potential reasons for the limited use for  $\text{NO}_3^-$  in blueberry (Alt et al., 2107).

Characteristics of N-source preference have been tested primarily in northern highbush blueberry and to a lesser extent in rabbiteye and southern highbush blueberry which are better adapted to the southeast. Further, the relative abilities of the newer and emerging genotypes for N-source preference are not well known. This information is essential to better manage nutrient application in blueberry production. Here, we present data from a study to determine N-source preference of some emerging blueberry cultivars using a split-root system.

## **Materials and Methods**

### *Plant Material*

One-year old 'SuziBlue' southern highbush blueberry cuttings were used for this study. The cuttings were obtained in February 2019 from Alma Nursery and Blueberry Farms in Alma, GA. The cuttings were transplanted into 1.6 L pots containing Fafard 3B growing mix and were grown out for 1-month after transplanting to ensure proper root formation and to maintain steady nutrition prior to the study. Plants received 100 ppm-N from a 20-20-20 All Purpose Peter's Professional Water-Soluble fertilizer via a Dosatron with a dilution rate at 1%.

### *Hydroponics System*

A split-root hydroponic system was constructed using 4-inch PVC tubing. The PVC tubing was cut into 15 cm cylinders that were 10 cm wide, allowing for a solution holding capacity of approximately 1.2 L per container. PVC end caps (10 cm) were then glued (pipe glue) to one end of the cylinder to form a 'cup'. On the open end of the cylinder, another 10 cm PVC end cap was loosely placed onto the cylinder. This end cap had a 'V' cutout to allow for roots of the plant to pass through the lid into the cylinder. A hole was drilled into the 'V' cut PVC end cap to allow for airline tubing and an airstone to be passed through. Forty-eight 'cups' were prepared and arranged on the greenhouse bench to allow for 24 research plants. Four air pumps were placed in the middle of the

greenhouse bench. Each pump was connected to an air manifold containing 12 ports. Airline tubing and an airstone was connected to each manifold port and run to each 'cup' in the experiment. One pump supplied air to one block of the experiment and there were 4 blocks with 4 pumps. The system was setup as a randomized complete block design with 4 blocks.

#### *Plant Positioning into Split-Root System*

Twenty-four uniform 'SuziBlue' cuttings were selected for this study. The plants were removed from the 1.6 L pot and the roots were washed extensively by hand to remove residual soil particles. Once the soil was removed from the roots, the plants were placed into the split-root hydroponics system. The roots of each cutting were evenly split between each 'cup'. Half of the root zone was placed into one 'cup' and the other half of the root zone was placed into a second 'cup'. Once the plants were placed in the system, the two 'cups' were covered with aluminum foil to reduce any evaporation from the containers.

#### *N Supply Treatments*

Once the plants were placed into the split-root hydroponics system, nutrient solution was added. A modified Hoaglands Solution was used for this study (Poornachit and Darnell, 2004). The 24 plants were first subjected to an acclimation period of 7 d in this solution. The acclimation Hoagland Solution contained 0.5 mM potassium phosphate, 1.0 mM magnesium sulfate, 0.5 mM calcium chloride, 0.08 mM Fe-EDTA, 0.045 mM boric acid, 0.01 mM manganese sulfate, 0.01 mM zinc sulfate, 0.02  $\mu$ M sodium molybdate, and 0.05 mM ammonium sulfate. A concentrate of the prepared Hoagland Solution was added to de-ionized water and the final volume of each 'cup' was adjusted to 1 L. After the acclimation period, treatments were applied for a 5 d period. The treatments consisted of supplementing the modified Hoagland's solution with N in the form of nitrate or ammonium. Further, some of the treatments involved supply of the nutrient solution differentially to each half of the split-root system. The Hoagland solution was identical to above, except for the modifications to the N source and concentration. The treatments were (Fig. 1): **1.** 50  $\mu$ M potassium nitrate to both cups of the split-root system; **2.** 500  $\mu$ M potassium nitrate to both cups of the split-root system; **3.** 25  $\mu$ M ammonium sulfate to both cups of the split-root system; **4.** 250  $\mu$ M ammonium sulfate to both cups of the split-root system; **5.** 50  $\mu$ M potassium nitrate to one half and 25  $\mu$ M ammonium sulfate to the other half of the split-root system; and **6.** 500  $\mu$ M potassium nitrate to one half and 250  $\mu$ M ammonium sulfate to the other half of the split-root system. After the treatment period, plants were removed from the system and allowed to air dry for 2 hours. After 2 hours, fresh weights were collected. The roots in each half of the split-root system of each plant were measured separately. The roots and

shoots were then placed in a drying oven for 5 d at 65°C. After drying, dry weights were collected and used for data analyses.

### *Sample and Data Collection*

Nutrient solution samples (45 mL) were collected at the start of the study. These samples were used to quantify the initial concentrations of N in our solutions. After the 5 d treatment, the remaining volume of solution in each ‘cup’ was measured and another 45 mL sample was collected to quantify the remaining N-concentration in each ‘cup’. The quantification of N in each sample was conducted spectrophotometrically using an auto-analyzer (Cabrera et. al., 2005). These data were used to determine the N quantity ( $\mu\text{mol}$ ) left in the ‘cup’ at the end of the experiment. The difference in N quantity at the beginning and the end of the experiment were used to determine the N uptake rate (per day). Further, these data were normalized to the root dry weight (root dry mass in respective ‘cup’) to correct for differences in root mass influencing N uptake. On the final day of the study, Chlorophyll Content Index (CCI) was collected using an Apogee CCI meter. Quantum yield (QY) of fluorescence was determined using a Fluorpen FP100. Electron Transport Rate was also calculated using the equation:  $\text{ETR} = \text{QY} \times \text{PAR} \times 0.84 \times 0.5$ ; where PAR (photosynthetically active radiation) was collected using a LiCor Light meter placed directly above the canopy of the plant.

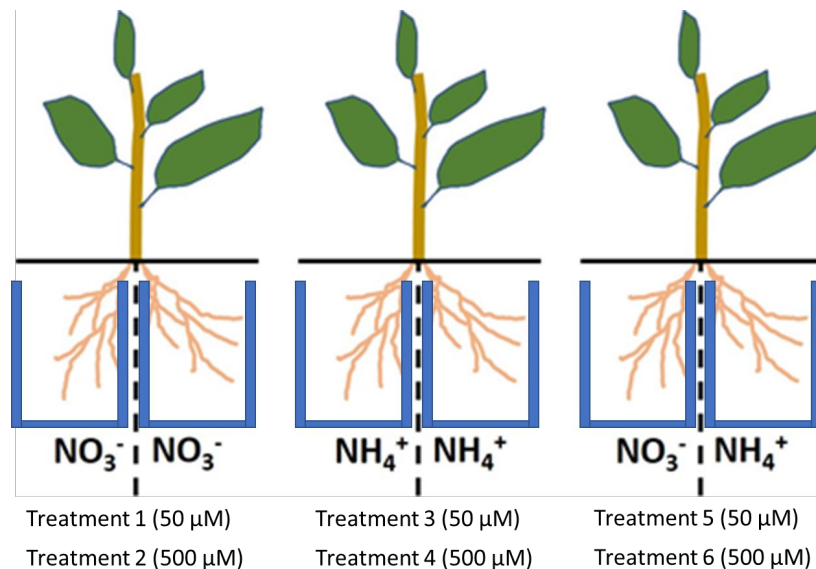
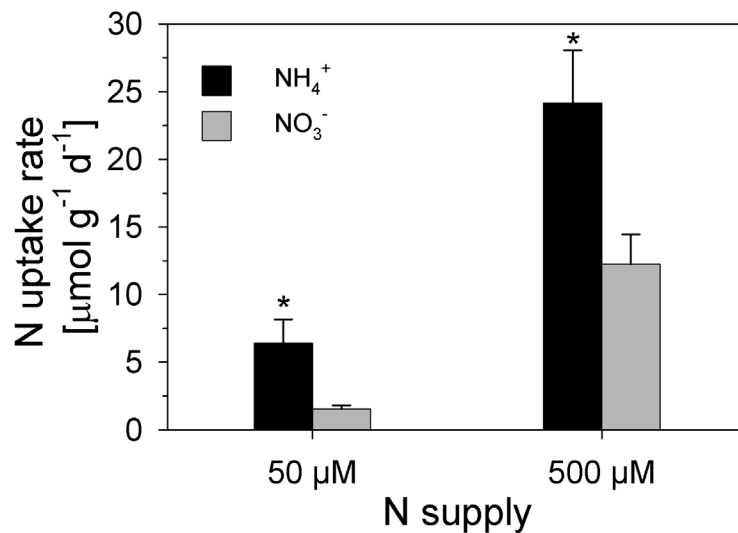


Fig. 1. A diagrammatic representation of the proposed split-root system treatments. Each segment of the roots will receive a specific source of N.

## Results and Discussion

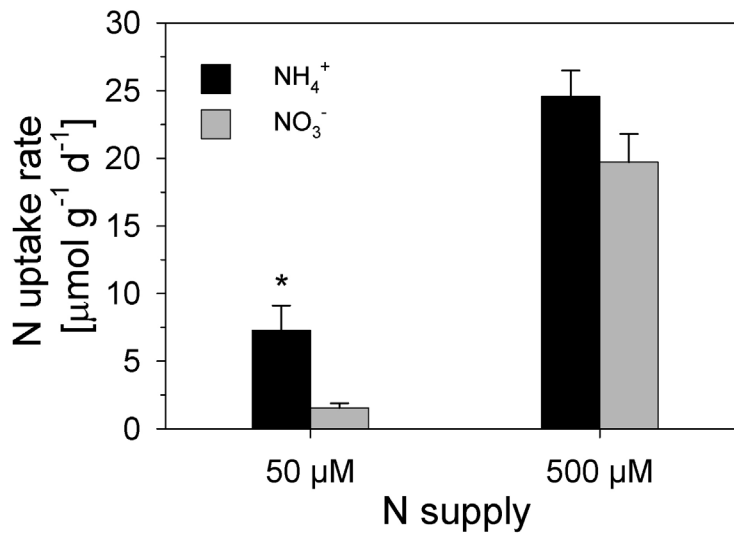
N-source preference was investigated at two different levels of N: 50  $\mu\text{M}$  and 500  $\mu\text{M}$  to determine if the rate of N-supply influences transport processes. At the low level of N supply (50  $\mu\text{M}$ ), clear differences in the N-uptake rates were observed. With  $\text{NH}_4^+$  as the N-source, the uptake rate was 6.4  $\mu\text{mol/g/d}$  while that under  $\text{NO}_3^-$  supply was around 3.6-fold lower at 1.8  $\mu\text{mol/g/d}$  (Fig. 2). These data indicate that the  $\text{NH}_4^+$  form of N was preferred under low N availability in blueberry. This is consistent with previous reports of N-source preference in blueberry. At higher N availability (500  $\mu\text{M}$ ), N uptake rate increased more than 3-fold under  $\text{NH}_4^+$  supply to 24.1  $\mu\text{mol/g/d}$  (Fig. 2). Under  $\text{NO}_3^-$  supply, the rate increased to 12.2  $\mu\text{mol/g/d}$ , around 2-fold lower. Together, these data indicate that ‘Suziblue’ blueberry displays N-source preference for the  $\text{NH}_4^+$  form at low and high levels of N-supply. However, the fold difference in uptake was substantially greater at the lower range in N-supply.



**Fig 2.** Nitrogen (N) uptake rate in response to different N forms and rates. Means and standard error ( $n = 4$ ) are shown. Data within a N-supply level were compared across the N-supply forms using the Student’s t-test. Asterisk indicates significant difference between the N-source forms within a given supply level.

Comparison of N supplied differentially to the split roots was performed. At the lower rate on N-supply, N uptake rate with  $\text{NH}_4^+$  supply was 7.3  $\mu\text{mol/g/d}$  (Fig. 3). This was comparable to that noted with similar level N-supply to both units of the split-root system. However, the rate of N uptake with  $\text{NO}_3^-$  supply was 4.6-fold lower than that under  $\text{NH}_4^+$  supply within the same set of plants under the split-root system (Fig. 3). Although N uptake rates increased substantially at higher N-supply, they were no longer significantly different between the two sources of N at this higher rate (Fig. 3). Together,

these data suggest that the rate of N-supply affects N-source preference in blueberry. At lower N-supply, a saturable high affinity transport system (HATS) is functional in most plants. Data presented here suggest that N-source preference for  $\text{NH}_4^+$  is displayed within the range where the HAT systems for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are functional. Hence, when the N-availability is low, the  $\text{NH}_4^+$  form may serve as the preferred source in blueberry. At higher concentrations of N, the low affinity transport systems are functional for both  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . At this range, comparison of N uptake in treatments supplied with only one form of N, indicated higher uptake rate for  $\text{NH}_4^+$ . However, the split-root treatments receiving both forms of N did not display a significant difference in N uptake rates. These data suggest that the preference for N-source may not be strongly expressed when N-availability is substantially high.



**Fig 3.** Nitrogen (N) uptake rate in response to different N forms and rates. Nitrogen forms were differentially supplied to a split-root system. One half of the roots received nitrate ( $\text{NO}_3^-$ ) while the other half received ammonium ( $\text{NH}_4^+$ ). These treatments were performed at two N-supply levels. Means and standard error ( $n = 4$ ) are shown. Data within a N-supply level were compared across the N-supply forms using the Student's t-test. Asterisk indicates significant difference between the N-source forms within a given supply level.

Several photosynthetic parameters were evaluated at the end of the study. The data indicated no significant differences across the treatments with respect to the chlorophyll content index, quantum yield or the photosynthetic electron transport rates (Table 1).

**Table 1.** Effect of nitrogen (N) source and concentration on photosynthetic parameters in ‘Suziblue’ southern highbush blueberry.

<b>Treatment</b>	<b>Quantum yield</b>	<b>Electron transport rate</b> <b>(<math>\mu\text{mol m}^2 \text{s}^{-1}</math>)</b>	<b>Chlorophyll content index</b>
<b>NO<sub>3</sub><sup>-</sup> (Low)</b>	0.58 ± 0.13	220.0 ± 97.3	24.6 ± 5.6
<b>NO<sub>3</sub><sup>-</sup> (High)</b>	0.48 ± 0.11	187.7 ± 76.1	20.6 ± 15.2
<b>NH<sub>4</sub><sup>+</sup> (Low)</b>	0.50 ± 0.10	192.1 ± 91.1	13.9 ± 6.6
<b>NH<sub>4</sub><sup>+</sup> (High)</b>	0.40 ± 0.08	178.6 ± 39.6	16.6 ± 7.0
<b>NO<sub>3</sub><sup>-</sup>/ NH<sub>4</sub><sup>+</sup> (Low)</b>	0.47 ± 0.12	201.5 ± 52.6	20.2 ± 13.4
<b>NO<sub>3</sub><sup>-</sup>/ NH<sub>4</sub><sup>+</sup> (High)</b>	0.58 ± 0.06	151.5 ± 93.0	12.1 ± 7.5
<b>ANOVA</b>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Together, the above data suggest that blueberry plants display N-source preference, particularly under low N-availability. These data suggest that the HAT systems functional for NH<sub>4</sub><sup>+</sup> may be more efficient than that for NO<sub>3</sub><sup>-</sup>. These data need to be further validated using other blueberry cultivars and rabbiteye blueberry genotypes. Further, a note of caution needs to be presented for the interpretation of the above data. During the analysis of N concentration in the hydroponics media, it was observed that control nutrient solutions (without added N) displayed trace amounts of NH<sub>4</sub><sup>+</sup>. Evaluation of traces in the individual salts used for the solution indicates trace contamination in several salts for this form of N. Hence, it is possible that the N supplied in the NH<sub>4</sub><sup>+</sup> treatment was higher and that the NO<sub>3</sub><sup>-</sup> sources also carried trace levels of NH<sub>4</sub><sup>+</sup>. We have currently identified salts without trace contaminations and will be performing repeat experiments in the future.



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