Progress Report

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Title: Analysis of uptake of nitrate and ammonium in blueberry using the ^{15}N stable isotope

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Public Abstract:

Blueberry is a major fleshy fruit in the southeastern United States. Sustaining the profitability of blueberry production requires optimization of management practices, including that of nitrogen (N) nutrition. Blueberry is considered to display N-source preference for the ammonium (NH_4^+) form of N over the nitrate (NO_3^-) form. The basis for this preference remains unclear. Further, N uptake characteristics at different external N concentrations are not well characterized in blueberry. In this study, we used the ^{15}N stable isotope to determine N uptake characteristics across a wide range of N concentrations, and across the two forms of inorganic N, NH_4^+ and NO_3^-. We determined that at lower concentrations of N, NH_4^+ and NO_3^- uptake was mediated by respective high affinity transport systems (HATS) which were saturable. At higher N concentrations, N uptake was mediated by distinct low affinity transport systems (LATS). The LATS for NH_4^+ uptake could be best described using a linear relationship. However, the LATS for NO_3^- uptake was saturable. Further, under HATS, the V_{max} associated with NO_3^- uptake was substantially lower than that for NH_4^+ uptake, suggesting lower root NO_3^- uptake capacity in blueberry. This may likely contribute to N-source preference. The current preliminary data presented in this report are based solely on uptake of ^{15}N into roots. Further analyses are underway to determine ^{15}N uptake/transport into shoots. Together these data are expected to provide comprehensive information on N uptake characteristics over a wide range of N concentrations in blueberry. Such information will be valuable in fine-tuning N nutrition management practices.

Objectives: The main objective of the proposed research is:
To determine nitrogen (N) uptake characteristics in blueberry using the ^{15}N stable isotope

Justification and Description:

Blueberry (*Vaccinium* species) has emerged as a major fruit crop in the Southeastern US. It is cultivated in over 30,000 acres in Georgia. The two major types of blueberry grown in Georgia are rabbiteye and southern highbush blueberry. Together, their production had a farm gate value

of over $300 million in 2018 (2018 Georgia Farm Gate Value Report). To ensure the continued profitability of this specialty crop in the southeastern US, considerable progress needs to be made in several areas of its production. One such aspect where research efforts need to be focused is blueberry nutrition. Blueberry belongs to the Ericaceae family. Multiple members of this family display specific nutritional requirements. These include a potential preference for the ammonium (NH_4^+) form over the nitrate (NO_3^-) form of inorganic nitrogen (N) (Townsend, 1969; Claussen and Lenz, 1999; Poonnachit and Darnell, 2004; Alt et al., 2017). Blueberry plants are also considered to be calcifuges, in that they are lime avoiding and prefer acidic soils (Korcak, 1988). Further, they are thought to prefer soils low in Ca^{2+}, although this may be associated with a preference for low pH soils. While most agricultural soils have higher NO_3^- levels, the low pH soils in which blueberry plants are often cultivated generally have higher availability of the NH_4^+ form of inorganic N. In particular, the preference that blueberry plants may display for the form of N (N-source preference) is of consequence to blueberry production. To ensure precise and effective nutrition of blueberry plants, it is essential that the right form of N be applied to the plants at the right amount. Such applications can contribute to the sustainability of blueberry production. In spite of its significance, many aspects of blueberry N nutrition are still poorly understood. Further, much of the knowledge on blueberry N nutrition is derived from older studies in northern highbush blueberry. Specific information on N nutrition in southern highbush and rabbiteye blueberry is still lacking.

The N concentration in blueberry leaves is typically at around 2% of the dry weight (Korcak, 1988). The N concentration is a result of multiple processes such as acquisition from the soil, its translocation to the shoots, and in case of mature plants, remobilization from stored reserves within the plant. Acquisition from the soil can occur in one of two forms of inorganic N: NO_3^- and NH_4^+. While most plants appear to prefer the NO_3^- form of N, blueberry and other Ericaceae members are considered to prefer the NH_4^+ form of N (Clausen and Lenz, 1999; Britto and Kronzucker, 2013). Acquisition of many nutrients including NO_3^- and NH_4^+ is facilitated by transporters, proteins involved in mediating the transfer of the solute across a membrane. In many plants, acquisition of NO_3^- and NH_4^+ has been demonstrated to occur in a bi-phasic manner (Aslam et al., 1992; Glass and Siddiqui, 1995; Min et al., 2000). For example, at lower concentrations of available NO_3^-, the transporter proteins involved in its uptake need to have a higher affinity to bind to the NO_3^- present in low concentrations and mediate its uptake into the cell. Hence, these proteins are considered to facilitate a high-affinity transport system (HATS) for NO_3^- uptake. At higher concentrations, proteins involved in NO_3^- uptake may display lower affinity for the substrate and can mediate its uptake. They are hence, facilitating a low-affinity transport system (LATS). HATS and LATS have been previously identified in many other plants for multiple nutrients including NO_3^-, NH_4^+, PO_4^{3-}, and K^+. The kinetics of HATS and LATS may differ substantially. HATS is often saturable and operational up to around 0.1 to 1 mM (for NO_3^- and NH_4^+; Aslam et al., 1992; Glass and Siddiqui, 1995; Min et al., 2000). In many cases, LATS displays a linear response to increasing concentration of the available nutrient. While it is likely that HATS and LATS are operational for NO_3^- and NH_4^+ acquisition in blueberry, they have not yet been clearly identified and described. This information may have practical implications for N nutrition in blueberry. It may be likely that blueberry plants prefer the NH_4^+ form of N within the HATS range of available N but they may not display such a response within the LATS range. This can potentially influence the form of N nutrition in blueberry production. It is also likely that differences in HATS kinetics influences the N uptake capacity of a given

type or cultivar of blueberry. The goal of this work is to determine the HATS and LATS N uptake characteristics of blueberry.

Materials and Methods:

Plant Material

'Suziblue' blueberry cuttings were purchased from Cornelius Blueberry Farms, Manor, GA and transported to the Riverbend Greenhouse Complex in Athens, GA in early 2021. The plants were then transplanted into 1-gallon containers filled with a 1:1 mixture of pine bark mulch and peat moss. Plants were fertigated weekly with J.R. Peter's Acid Special (21-7-7) at 50 ppm N. Cuttings were grown out for 1 month to allow for proper root development. Three days prior to exposure to hydroponics, plants were not watered to allow for optimal root washing and removal of substrate.

Hydroponic System and Modified Hoagland's Solution

The hydroponic system consisted of 1-quart plastic containers covered in tinfoil to reduce light penetration into the container. The lids on the container had a "X" cut into them to allow for insertion of plant roots into the container. One plant was placed into each cup and suspended from a trellis system in the greenhouse. The root collar of the plant was suspended 1´´ above the solution in the paint bucket. Aeration was supplied to each paint bucket via an air pump and airline tubing with an air-stone.

Four individual studies were conducted to investigate HATS (NH_4^+/NO_3^-) and LATS (NH_4^+/NO_3^-) uptake kinetics in blueberry. Plants used in all investigations were first exposed to an acclimation solution for 5-d, followed by a starvation solution for 2-d. The foundation of the hydroponic solution was a modified version of Hoagland's hydroponic solution and consisted of 0.5 mM potassium phosphate, 1 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, and 0.02 µM sodium molybdate. Nitrogen-source was supplied as either ammonium sulfate or potassium nitrate. The acclimation solution consisted of non-labelled N-sources and the treatment solution consisted of labelled ^{15}N-sources. The acclimation solution for HATS had a concentration of 250 µM N and the starvation solution concentration was 0 µM N. There were seven treatment solutions for HATS with concentrations of 5.075, 10.15, 25.37, 50.75, 75.12, 101.49 and 253.74 µM $(^{15}NH_4)_2SO_4$, 3.14 atom % enrichment. Nitrate HATS treatment concentrations were 5, 10, 25, 50, 75, 100 and 250 µM $K^{15}NO_3$, 5 atom % enrichment. Plants used for LATS investigation were exposed to the same schedule of acclimation, starvation, and treatment solutions. The acclimation solution for LATS consisted of 1 mM N and the starvation solution concentration was 0 mM N. There were seven treatment solutions supplied to investigate LATS uptake kinetics with concentrations of 0.102, 0.508, 1.02, 2.54, 5.08, 10.15, and 25.37 mM $(^{15}NH_4)_2SO_4$, 3.14 atom % enrichment and 0.1, 0.5, 1, 2.5, 5, 10 and 25 mM $K^{15}NO_3$, 5 atom % enrichment. Treatment duration was 6-h for the LATS analyses and 24-h for the HATS analyses.

Sample Collection and Preparation

Root and shoot samples were collected at the end of the experiment. Root samples were washed three times; the first and second wash consisted of the modified Hoagland's solution with non-labelled N-sources, and the final wash consisted of the modified Hoagland's solution without N. Root wash timeframes were kept consistent between washes and among root samples. Washes lasted < 1 min and were conducted to remove any labeled N-sources adhering to the surface of

the root tissue. Once roots were washed, they were dried and placed into 50 mL tubes. Shoot samples were collected and entire sample was placed into 50 mL tubes. All sample tubes were pre-frozen in liquid Nitrogen with care taken not to contaminate the tubes and then re-frozen once sample was in place. Samples were stored at -80 ^{o}C until freeze drying for > 24 h. After freeze drying, samples were submitted to the Stable Isotope Ecology Laboratory at the Center for Applied Isotope Studies at the University of Georgia, Athens, GA (SIEL-UGA) where they were prepared and processed for analysis.

Isotope Ratio Mass Spectrometry Analysis

After freeze-drying, samples were finely ground and 2-3 mg (root tissue) was added to encapsulation tins after weighing on a semi-microbalance accurate to 0.01 mg. These tins were then placed in a 96-well microtiter plate and submitted for analysis. Analysis consisted of combusting the encapsulated samples at 1100 ^{o}C and the gases produced were delivered via continuous-flow for analysis of $\delta^{15}N$ using isotope ratio mass spectrometry (IRMS) where enrichment of samples was compared to natural abundance of ^{15}N in the atmosphere (0.37 atom %). Results were presented as atom % ^{15}N and this was used to calculate to Atom % Excess (APE) for each sample. The APE was then used to calculate ^{15}N incorporated (during acquisition) into the plant root tissue. The analysis was conducted by using the samples with the least amount of enrichment first and moving to samples with the highest enrichment to reduce contamination within the lab setting. All materials used for the analysis were thoroughly cleaned with ethanol between each sample to further reduce chances of contamination.

Experimental Design

These studies were conducted as randomized complete block designs with 4 replicates ($^{15}NH_4^+$) and 5 replicates ($^{15}NO_3^-$). Uptake rates were calculated based on APE compared to control plants not supplied with ^{15}N. Figures were generated using JMP software and statistical analysis was conducted using R open-source software and JMP software.

Results:

Ammonium uptake in blueberry

Ammonium uptake in blueberry displayed saturable kinetics within the low N concentration range indicating saturable HATS for its uptake. The plot of the rate of ^{15}N uptake against the substrate (N) concentration could be described using Michaelis-Menten kinetics (Fig. 1; R^2 = 0.71; P = 0.0001). The K_m of the $^{15}NH_4^+$ uptake system operating at low N concentrations (HATS), was 35.04 µM while the V_{max} was 2.26 µmol ^{15}N g $(dw)^{-1}$ d^{-1}. Within the high concentration range corresponding to LATS, $^{15}NH_4^+$ uptake rate increased initially with increasing N concentration and appeared to decline at higher concentrations. However, comparisons across various curve fitting models indicated that the kinetics could be best described using a linear relationship (R^2 = 0.97). These data suggest that the LATS for NH_4^+ uptake increased linearly with increasing N concentration, without saturation in the range of N concentrations studied (up to 50 mM). Together, these data indicate distinct HATS and LATS uptake mechanisms for NH_4^+ uptake in blueberry. The HATS displayed saturation kinetics with around 80% of V_{max} achieved by around 100 µM N. The LATS could be best described using a linear relationship.

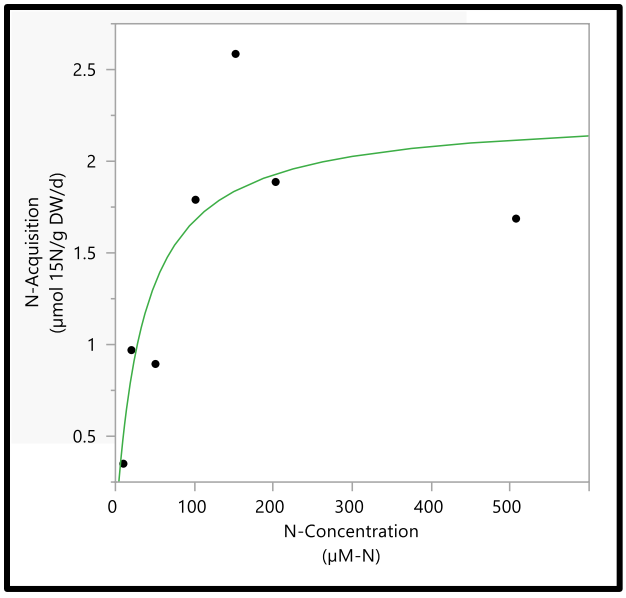

Fig. 1. Ammonium uptake kinetics associated with HATS in 'Suziblue'. Ammonium uptake kinetics was monitored by quantifying ^{15}N uptake into roots. Nitrogen was supplied as $(^{15}NH_4)_2SO_4$. $R^2 = 0.71$; $P = 0.0001$.

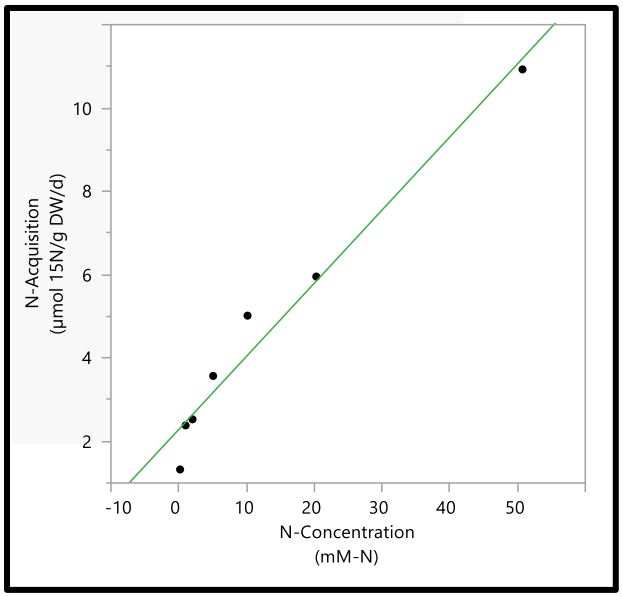

Fig. 2. Ammonium uptake kinetics associated with LATS in 'Suziblue'. Ammonium uptake kinetics was monitored by quantifying ^{15}N uptake into roots. Nitrogen was supplied as $(^{15}NH_4)_2SO_4$. $R^2 = 0.97$.

Nitrate uptake in ‘Suziblue’ blueberry roots displayed a clearly saturable HATS within the range of 5 -250 µM N (Fig. 3). Michaelis-Menten kinetics analysis (R^2 = 0.81; P = 0.0001) indicated a K_m of 15.94 µM and a V_{max} of 0.32 µmol ^{15}N g (dw)$^{-1}$ d^{-1} for the NO_3^- HATS. Further, over 97.5% of the V_{max} was attained at a substrate concentration of around 100 µM N. At higher concentrations, distinct and saturable LATS for NO_3^- uptake was evident (Fig. 4). Curve fitting analysis indicated that Michealis-Menten kinetics could best describe the LATS (R^2 = 0.94; P = 0.0001). Further, these analyses indicated a K_m of 4.52 mM and a V_{max} of 1.41 µmol ^{15}N g (dw)$^{-1}$ d^{-1} for the NO_3^- LATS.

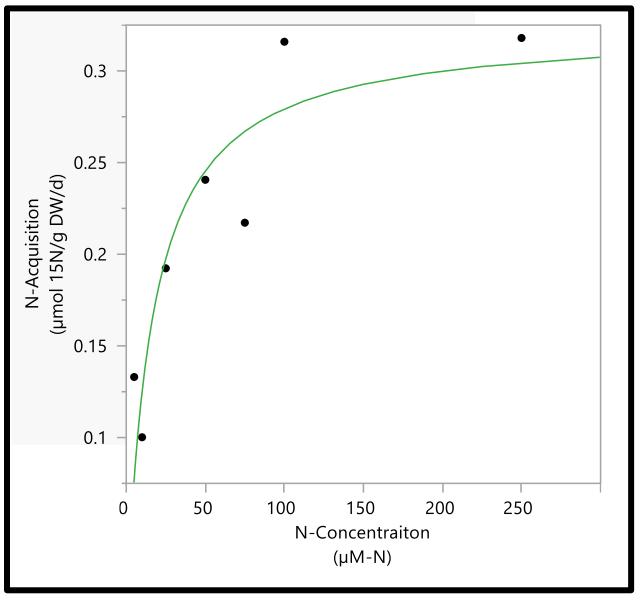

Fig. 3. Nitrate uptake kinetics associated with HATS in ‘Suziblue’. Nitrate uptake kinetics was monitored by quantifying ^{15}N uptake into roots. Nitrogen was supplied as K^{15}NO$_3$. R^2 = 0.81; P = 0.0001.

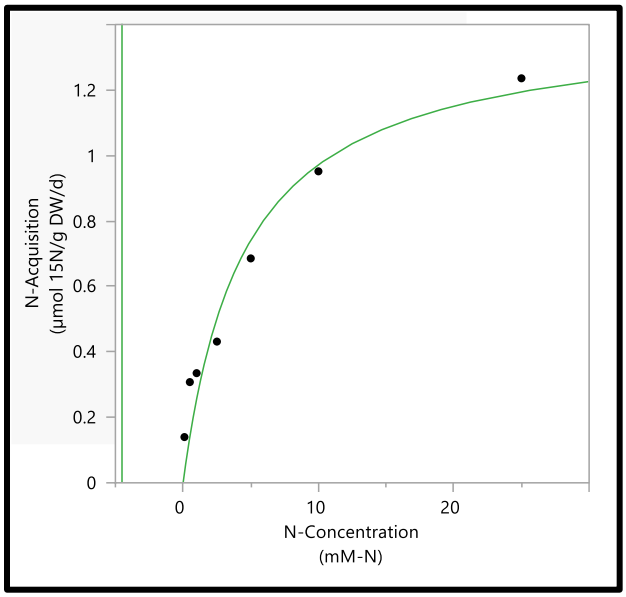

Fig. 4. Nitrate uptake kinetics associated with LATS in ‘Suziblue’. Nitrate uptake kinetics was monitored by quantifying ^{15}N uptake into roots. Nitrogen was supplied as K^{15}NO$_3$. R^2 = 0.94.

Together, these data indicate saturable HATS and LATS associated with NH_4^+ and NO_3^- uptake in blueberry. Interestingly, the K_m of the HATS was around 2-fold lower for NO_3^- uptake than that for NH_4^+ uptake, while the V_{max} was 7-fold lower than that for NH_4^+ uptake. Further, the LATS for NO_3^- uptake was saturable unlike that noted for NH_4^+ uptake. These data suggest a substantially lower NO_3^- transport capacity into blueberry roots in comparison to that for NH_4^+ uptake. It may be speculated that these differences contribute to N-source preference in blueberry.

Significance

This study helped determine N-uptake characteristics in blueberry at different N concentration ranges involving HATS and LATS with greater sensitivity. These data indicate that the NO_3^- uptake capacity is lower than that for NH_4^+ uptake at a given N concentration, particularly in the range typically associated with blueberry production. Further, these data suggest that uptake may be an important aspect of N-source preference in blueberry. This information will be useful in optimizing N management strategies for southeastern blueberry production. Management practices can be specifically targeted such that the preferred N source is precisely provided at the right concentration range. This is expected to help increase productivity and profitability of blueberry production in the southeastern US.

Future Work

An important note of caution with interpretation of the current work described above is that it is currently based on data obtained from ^{15}N uptake into root tissues only. We have collected shoot tissues from these experiments and are currently processing them for ^{15}N analyses using the same methods. These data need to be combined with that data presented above to obtain comprehensive information on N uptake characteristics. Hence, data presented above should be considered preliminary.

References

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