Public Abstract
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Title: Screening of current southeastern blueberry cultivars for ammonium and nitrate uptake capacities

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Blueberry has emerged as a major fruit crop in the southeastern US. Blueberries are thought to prefer the ammonium form of nitrogen (N) over the nitrate form. The physiological basis of such preference is not completely clear, although one potential reason for such a preference is the differential uptake of N by blueberry roots. However, it is not clear if currently grown blueberry genotypes in the southeastern US display preferences in the uptake for different forms of N. Additionally, the extent of such preference across currently grown genotypes remains unclear. The goal of the current study was to determine N uptake rates across currently grown cultivars. 'Farthing' and 'Brightwell' blueberry plants were used for this study in a hydroponics-based system. These plants were subjected to N in the form of nitrate and ammonium. Each of these N sources were provided at a low and a moderate concentration. To determine the N uptake rate, a naturally occurring stable isotope of N, ^{15}N, was used as a tracer. Over a short period of acclimation and experimentation, neither the form of N nor the N concentration significantly affected root and shoot dry weights in both cultivars. Determining N-uptake using ^{15}N requires specialized analyses performed by only three laboratories in the US, with one of them at the University of Georgia. As this lab is temporarily not accepting new submissions for stable isotope analysis, the N-uptake analysis could not be completed. The lab will resume analyses in Spring 2024, at which time ^{15}N abundance analysis will be completed to determine the extent of N-uptake across the blueberry genotypes.

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Objectives: The main objective of the proposed research is:
To determine the N-uptake capacities for ammonium and nitrate across major rabbiteye and southern highbush blueberry cultivars

Justification and Description:
Blueberry (*Vaccinium* species) has become a major fruit crop in the southeastern US, particularly in Georgia (GA). Currently, rabbiteye (RE) blueberry and southern highbush (SH) blueberry are the major types cultivated in GA. Blueberry was cultivated in over 26,000 acres and was valued at over $300 million in GA (2020 Georgia Farm Gate Value Report). Fine-tuning production practices is essential to sustain the current profitability of blueberry production. One set of production practices that requires further optimization in blueberry, is nutrient management, particularly that of nitrogen (N). Current N management practices are often based on older studies (and older cultivars) or those previously performed with Northern Highbush blueberry. Further, characteristics of N-uptake from the soil and its utilization within the plant are not well understood in currently grown blueberry genotypes in the southeast. Hence, information to make nutrient management decisions for current cultivars needs to be derived from studies on cultivars of significance to southeastern blueberry production.

Nitrogen is a macronutrient and significant efforts are often placed to optimize the availability of N in plant nutrient management. In blueberry leaves, optimal N concentration is typically between 1.76 and 2% (dry wt. basis; Bryla and Strik, 2015). Nitrogen deficiency in blueberry results in chlorosis of older leaves (yellowing) and substantial reduction in growth, particularly when it occurs during early plant establishment (Hart et al., 2006). Plants can take in N either in the organic forms (amino acids, peptides) or in the inorganic forms. Inorganic forms of

N are generally most abundant under typical agricultural production conditions. The two main inorganic forms of N are NH_4^+ and NO_3^-.

In relation to N-nutrition, blueberry plants display two specific characteristics: 1. A potential preference for the NH_4^+-form of N over the NO_3^--form; 2. A low pH requirement (acidic soils) where NH_4^+ is thought to be the predominant N-form. Both these factors influence N management practices. Preference for NH_4^+ results in the use of ammonium-based fertilizers for N-nutrition. A low pH-requirement often requires that typical production soils that have higher pH are amended, often extensively to allow for optimal blueberry plant growth.

As described above, blueberry is thought to display a preference for the NH_4^+ form of N over the NO_3^- form. However, there are multiple aspects of this preference (N-source preference) that are not clearly established. While some previous studies suggest that blueberry plants display such preference, others indicate that blueberry plants are capable of using both forms of inorganic N (Cain, 1952; Oertli, 1963; Claussen and Lenz, 1999; Poonnachit and Darnell, 2004; summarized in Doyle et al., 2021). As NH_4^+-based fertilizers are typically applied in blueberry production, it is expected that plants utilize only this form of N. However, recent studies suggest that even when NH_4^+ form of N is applied to acidic soils, certain microorganisms (Ammonia-Oxidizing Archaea) that are prevalent can nitrify NH_4^+ to NO_3^- (Hu et al., 2014). Hence, under field conditions, it is likely that blueberry plants are exposed to both forms of inorganic N even if only NH_4^+-based fertilizers are applied. Under such conditions, it is not clear if blueberry plants are still able to uptake and utilize the NO_3^- form of N. Further, it is not clear if different genotypes of blueberry, particularly current cultivars, display a preference for a specific form of N or if they are able to similarly uptake both forms. In other plant species that primarily utilize NH_4^+, considerable variability exists across genotypes, particularly in the uptake of different N-forms (Britto and Kronzucker, 2013; Hu et al., 2015). Such variability has not been evaluated across blueberry genotypes.

Plants use different mechanisms to acquire N depending on the concentration of available N. At low to moderate external N concentrations (< 0.1 mM), plants use high affinity transport systems (HATS) which display greater affinity but an overall low capacity for N-uptake. At higher concentrations (> 1mM), plants utilize low affinity transport systems (LATS) which have relatively lower affinity for N but a greater capacity (Glass et al., 2002). A previous study in blueberry (funded by SRSFC in the PI's lab) indicated that blueberry plants also display similar mechanisms. Further, this study also indicated that blueberry plants display a greater capacity for NH_4^+ uptake (compared to NO_3^-) when external N concentration is low-moderate (< 0.25 mM). At higher external N, the uptake rates, as mediated only by LATS, were comparable for NH_4^+ and NO_3^-. This study was performed only in one SH blueberry cultivar ('Suziblue'), and it remains to be determined if these characteristics are similar across other genotypes and other blueberry species.

Evaluation of the N-uptake capacities across blueberry genotypes has not been performed previously. Determining how different blueberry cultivars deal with N-availability in the field can help us to fine-tune N-management practices in blueberry production. Hence, the main goal of this study is to evaluate the uptake capacities for NH_4^+ and NO_3^- for different RE and SH blueberry genotypes. Particularly, the objective here is to determine such capacities in cultivars currently grown in GA and in the southeast. Additionally, the focus of the current study is to evaluate the NH_4^+ and NO_3^- uptake capacities of these cultivars under conditions of low-moderate N availability, a range wherein previous studies indicate potential differences.

Materials and Methods

Plant material and Hydroponics Set-up

Two cultivars commonly cultivated in the southeastern US were used for the initial phases of this trial. The southern highbush cultivar, 'Farthing' and the rabbiteye cultivar 'Brightwell, were utilized. Plants were purchased as 1-year old cuttings from a nursery in south GA. Plants were grown in a greenhouse at the University of Georgia, Athens until utilized for the experiment. A hydroponics system previously developed in the PI's laboratory was used to determine N uptake rates in these two cultivars. Plants were removed from the pots and the roots were carefully washed multiple times to remove substrate adhering to the root system. Subsequently, these plants were introduced into the hydroponics system which consisted of 20, 0.8 L containers procured from a local hardware store. Plants were suspended using a wire trellis system with their roots immersed within the solution in the container (Fig. 1). The rootzone collar of the plants was around 2.5 cm above the hydroponics solution. Air pumps connected to plastic tubing were used to provide constant aeration to the root zone by bubbling ambient air through the solution.

Hydroponics Solution

A modified Hoagland's solution was used to provide nutrients to the plants through the hydroponics system. The composition of the solution was as follows: 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, 0.02 µM sodium molybdate. Nitrogen at the target concentration was provided either as potassium nitrate (KNO_3), or as ammonium sulfate [$(NH_4)_2SO_4$]. Plants were initially subjected to an acclimation period of 8 d to allow the plants to adjust to the hydroponics conditions. During the period of acclimation, plants received the N concentration corresponding to the N levels they were subsequently subjected to for uptake analysis. The pH of the nutrient solution was maintained around 5.0 through addition of HCl. Two complete changes in nutrient solutions were performed during the acclimation period, at 2 d and 5 d after initiation. At each change of nutrient solution, the pH was adjusted to the required level of 5.

Treatments

Four treatments were used in this study to evaluate N uptake. 1. 50 µM NO_3^-; 2. 500 µM NO_3^-; 3. 50 µM NH_4^+; and 4. 500 µM NH_4^+. Previous results from work in the PI's laboratory indicated that N uptake rates in blueberry depend on the level of externally available N. These concentrations were chosen to determine N uptake rates at low and medium levels of N availability. The experimental set-up was a randomized complete block design with five replications. To determine N uptake, the stable isotope of N, ^{15}N, was used. $K^{15}NO_3$ and $(^{15}NH_4)_2SO_4$ procured from

Cambridge Isotope Laboratories, Inc. (Tewksbury, MA) were used. The stable isotope labeled salts were provided by incorporating the required ^{15}N salt into the hydroponics solution at a final level of 3% atom percent and at the required N concentration. These treatments were provided at the end of the 8-d acclimation period. Plants were subjected to the ^{15}N source of N for a total duration of 18 h. At the end of the experiment, the plants were removed from the hydroponics solution and washed three times in de-ionized water for a duration of 1 min each. This was performed to remove any ^{15}N adhering to the root surface. Subsequently, the roots were dried by blotting with paper towels. The root and shoot tissues were separately harvested and placed in brown paper bags for drying. Tissues were oven dried at ~ 70 °C for several days until the plant material reached a constant dry weight. Subsequently, plant dry weights were obtained using a precision balance.

Results

Plant root and shoot dry weights were measureed. These data indicate responses to ~ 9 d of N supply for the four treatments. In 'Farthing' neither the N-source, nor the N concentration affected root and shoot dry weights (Fig. 2). Similarly, in 'Brightwell', neither the root dry weight, nor the shoot dry weight were significantly affected by the N treatments (Fig. 2). Together, these data indicate that N source and N concentration used here did not significantly alter root and shoot dry weight characteristics over the short duration of the experiment.

The main goal of this study was to determine the N uptake rates in the blueberry cultivars. The method chosen to assess N uptake was the use of ^{15}N tracer to determine the rate and extent of N uptake into the plants following the 18 h treatment period. Determination of the uptake rates requires that the plant samples are analyzed for ^{15}N abundance following its uptake. This is performed using an Elemental Analyzer (EA) followed by a Isotope Ratio Mass Spectrometer (IRMS). These specialized analyses of isotope ratios are performed only by three major facilities in the United States. Among these, the Stable Isotope Ecology Laboratory (SIEL) at the University of Georgia is a leading lab specializing in stable isotope analyses. The PI has previously developed ^{15}N determination protocols in blueberry tissues based on inputs from this lab. Subsequently, multiple analyses on ^{15}N-uptake in blueberry have been performed by the PI at this facility.

The PI intended to submit the samples from this study for analyses to the SIEL at UGA. However, this lab has recently undergone personnel changes and retirements. Further, the lab has been dealing with a backlog running into several months (personal communication). Hence, as of August 2023, this lab temporarily halted acceptance of further samples for processing. Please see: https://cais.uga.edu/facilities/stable-isotope-ecology-laboratory/ . Owing to the above reason, samples collected from this study could not be further processed for determining ^{15}N abundance in plant tissues. The samples were not sent to other US facilities that can perform stable isotope analyses as previous protocols developed by the PI were based on analyses performed at SIEL, UGA. The SIEL at UGA is likely to open acceptance of new samples from early Spring 2024 (personal communication). Once the lab starts accepting submissions, samples from this study will be submitted for ^{15}N abundance analysis. Additionally, further experiments will be conducted at that time with other cultivars and subjected to similar analyses. To allow for the processing of the samples and the continuance of the experiments, a no-cost extension of the current project will be requested. This will allow the PI's lab to complete the proposed experiments and process the samples for N-uptake analysis in 2024. Once these experiments and analyses are completed, a new progress report will be submitted to the SRSFC.

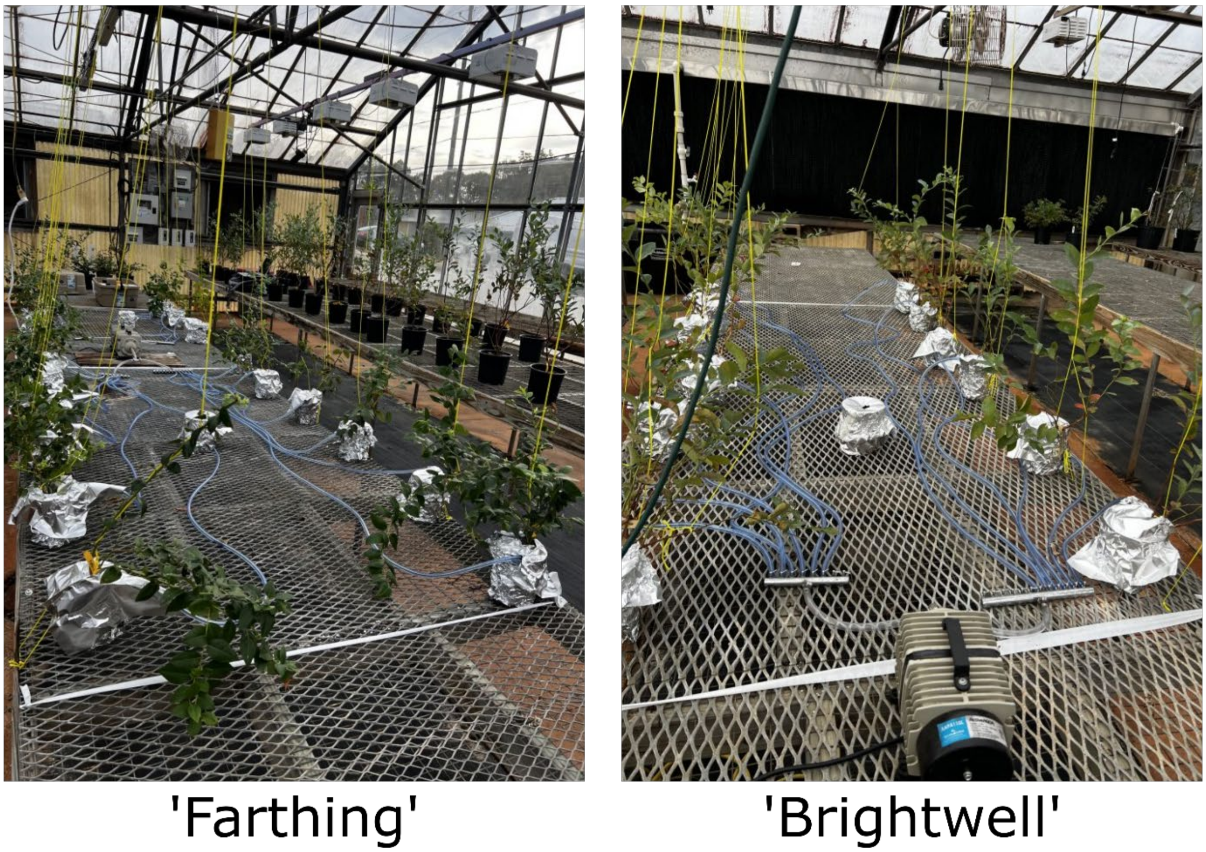

Fig. 1. Hydroponics experimental set up for N-uptake analysis in one southern highbush ('Farthing') and one rabbiteye ('Brightwell') blueberry cultivar.

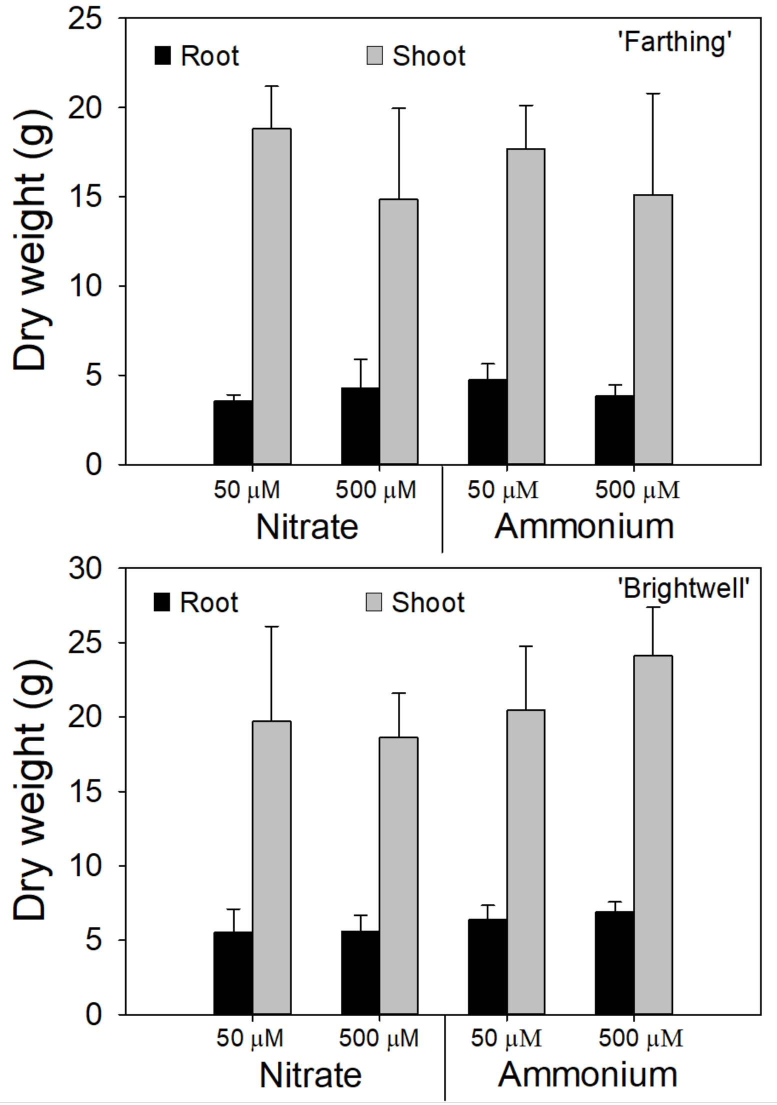

Fig. 2. Root and shoot dry weigh responses during acclimation and nitrogen (N) – uptake analysis duration in 'Farthing' and 'Brightwell' blueberry genotypes. Blueberry plants were subjected to acclimation with different N sources (nitrate and ammonium), each at two different N concentrations (50 µM and 500 µM). Neither the N-source nor the N-concentration affected root and shoot dry weights in the two blueberry genotypes.

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