Title: Evaluation of the Nitrogen Use Capacities of Emerging Southern Highbush and Rabbiteye Blueberry Varieties

Progress Report

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Objectives: The main objective of the proposed research is:

To evaluate the capacity of emerging blueberry varieties to acquire and utilize different forms of inorganic nitrogen

Justification and Description:

Blueberry (*Vaccinium* species) has rapidly emerged as a major fruit crop of high economic value in the Southeast over the past decade. In Georgia, over 28000 acres are currently under blueberry cultivation and the crop was valued at over 335 million dollars in 2014 (2014 Georgia Farm Gate Value Report), making it the major fruit crop in the state. Sustained profitability of blueberry production in Georgia and the Southeast requires that various aspects of its cultivation including nutrient management are systematically optimized through scientific efforts.

Nitrogen is a macronutrient that constitutes up to 2% of blueberry plant dry weight and plays various important roles in its growth and development. Its efficient management can therefore result in significantly improved productivity and profitability for the growers. Ammonium and nitrate are the two inorganic forms of nitrogen acquired and utilized by plants. Many of the cultivated blueberry species are generally thought to prefer ammonium as the main form of inorganic nitrogen (Townsend, 1969; Korcak, 1988; Poonnachit and Darnell, 2004). Reduced growth of blueberry plants grown with nitrate as the only source of nitrogen in comparison to those grown with ammonium alone has been reported previously (Townsend, 1969; Claussen and Lenz, 1999; Poonnachit and Darnell, 2004).

Many of the previous studies that indicated reduced growth under nitrate nutrition were performed using older varieties which are no longer used in Southeastern blueberry production. In the recent past, many new and promising southern highbush and rabbiteye cultivars have been introduced through breeding programs such as that of the Co-PI. It is currently unknown whether these newer varieties display specific preference for the ammonium form over the nitrate form of nitrogen, and whether they display better growth characteristics under the supply of ammonium form of nitrogen. Even with a preference for ammonium, it is likely that there is significant variability among these newer varieties for its acquisition and utilization. Further, it is not clear if southern highbush blueberries differ from rabbiteye blueberries in their nitrogen acquisition and utilization capacities. Knowledge in the above areas can be valuable in optimizing nitrogen management methods for Southeastern blueberry production.

The potential preference for ammonium over nitrate in blueberry may be due to a lower capacity for taking up nitrate from the soil, low translocation of nitrate to the shoots, or due to an overall lower capacity to assimilate nitrate (Dirr et al., 1972; Claussen and Lenz, 1999; Merhaut and Darnell, 1995; Poonnachit and Darnell, 2004; Darnell and Hiss, 2006; Alt et al., 2017). A key enzyme involved in nitrate assimilation is nitrate reductase (NR), which catalyzes the conversion of nitrate to nitrite which is subsequently converted to ammonium. Ammonium is incorporated into amino acids through the activities of glutamine synthetase (GS) and glutamate synthase. In blueberry, much of nitrate assimilation occurs in the roots while the leaves display a limited capacity for the same (Poonnachit and Darnell, 2004; Alt et al., 2017)

The goal of this project is to evaluate some of the newer and emerging southern highbush and rabbiteye blueberry cultivars for their ability to acquire and utilize different forms of inorganic nitrogen. Multiple southern highbush and rabbiteye blueberry varieties will be grown using a hydroponics system which allows for effective manipulation of the nutrients supplied. Growth, biomass accumulation, and nitrogen content and its metabolism will be studied in these varieties to determine their overall nitrogen use capacities.

Experimental Plan:

Plant Material and Experimental Set-up

Three southern highbush blueberry cultivars were used in the study: Star, Suziblue and Ms. Alice Mae. The cultivar Star, was selected to serve as a reference while the other two cultivars represented recently released southern highbush cultivars of emerging significance for southeastern blueberry production. The plants were initially maintained in 3-gallon pots with pine-bark based media to allow for their initial establishment. A hydroponics system established previously in the PI's research program for the evaluation of nutrient effects on blueberry growth and development, and physiology was used in this study (Alt et al., 2017). Uniform size plants were selected and transferred to the hydroponics system with the provision of the required nutrients through a nutrient solution as described in Alt et al., 2017. However, 'Suziblue' plants were generally smaller than the other two cultivars. The pH of the nutrient solution was maintained at around 5.0 by monitoring it two times per week. An initial period of acclimation was provided to allow for the plants to acclimate to the hydroponics system. Nitrogen was provided in the ammonium form [1 mM $(NH_4)_2SO_4$] during this period. Treatments involving the supply of two different inorganic forms of nitrogen was performed. The two treatments were: 1. Ammonium (as 2.5 mM Ammonium Sulfate); and 2. Nitrate (as 5 mM Potassium Nitrate). Four containers without plants were used to determine the extent of evaporative water losses. Other essential nutrients were provided in the required quantities as per Poonnachit and Darnell, 2004 and Alt et al., 2017. The nutrient solution was changed weekly. The experimental design was a randomized complete block design with four replications. Data analyses were performed using Analysis of Variance (ANOVA) in JMP.

Measurement of Growth, Nitrogen Acquisition and Nitrogen Utilization

At the beginning of the nitrogen treatments, one shoot per plant was tagged and monitored throughout the duration of the experiment to determine the effect of the supplied nitrogen form on blueberry growth. At the end of the experiment, plant fresh weight and dry weight data were collected for determining the accumulated biomass. At each change of the nutrient solution, the previous solution sample was collected. Nitrogen content left within these samples was measured spectrophotometrically using an auto-analyzer (Cabrera et al., 2005). Data for the ammonium content within the solution samples are currently available while the nitrate content is being analyzed. These data were used to determine the amount of nitrogen removed by the plants after correcting for water loss from the containers due to evapo-transpiration. Root (~0.2 g) and leaf samples (~0.2 g) were collected at weekly intervals during the experiment, frozen and stored. These samples will be used to determine the tissue nitrogen content and enzyme activities. At the end of the experiment, root and leaf samples were collected to determine their nitrogen contents and for enzyme activity measurements.

Results:

Shoot growth rate was measured over the duration of the experiment (up to 18 d after treatment). The rate of shoot growth was up to 2 mm per day over this duration (Fig. 1). Shoot growth rate was not statistically different among the three southern highbush cultivars (Fig. 1). Further, the N treatment did not significantly affect the rate of shoot growth, although there was a trend for lower shoot growth rates with nitrate as the source of N across the three cultivars studied.

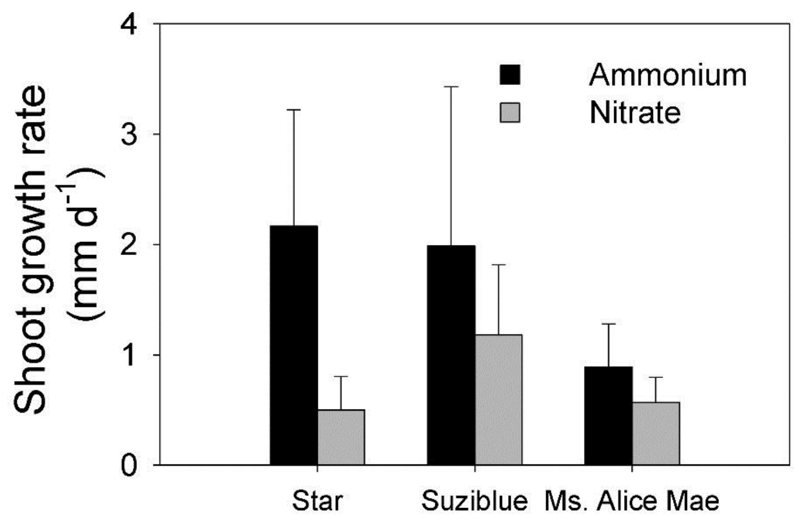

Fig. 1. Shoot growth rate in three southern highbush cultivars in response to ammonium and nitrate as sources of nitrogen. Shoots were measured during the duration of the experiment (18 d). Error bars represent the standard error of the mean (n = 4).

Shoot fresh weight and dry weight were significantly different across the three southern highbush cultivars (Figs. 2 and 3; P = 0.0005 and 0.0003, respectively). 'Suziblue' displayed lower shoot fresh weight and dry weight than that of both 'Star' and 'Ms. Alice Mae'. These differences may be in part due to differences at the onset of the experiment. The root fresh and dry weights were not significantly different among the three cultivars, although 'Suziblue' displayed a trend toward lower root weights (Figs. 2 and 3; P = 0.058 and 0.051, respectively). The N treatment did not significantly affect the fresh or dry weights of either of the organs.

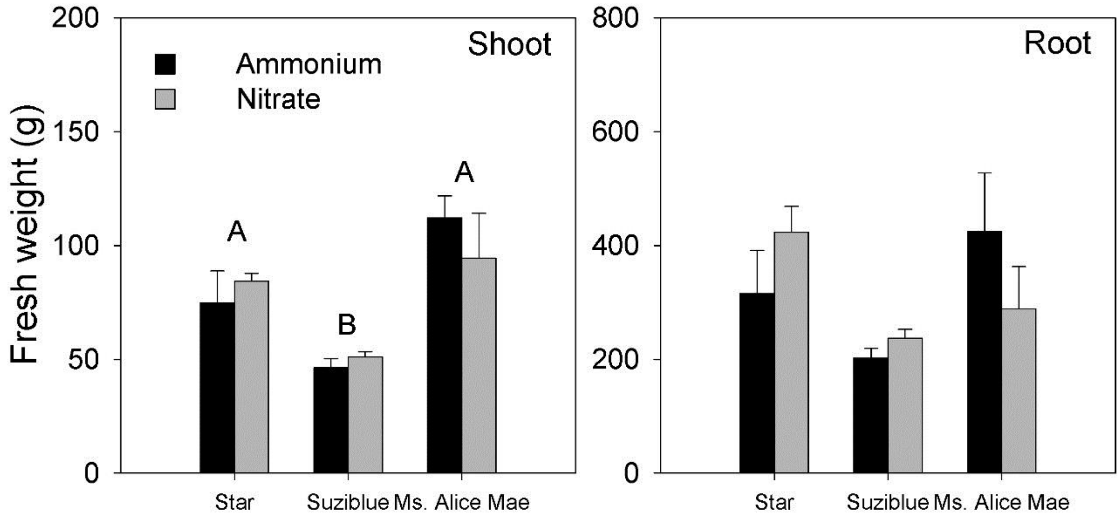

Fig. 2. Fresh weight of the shoots and roots in three southern highbush cultivars in response to ammonium and nitrate as sources of nitrogen. Weights were measured at the end of the experiment (18 d). Error bars represent the standard error of the mean (n = 4). Similar letters above the bars indicate that the cultivars were not significantly different ($\alpha = 0.05$).

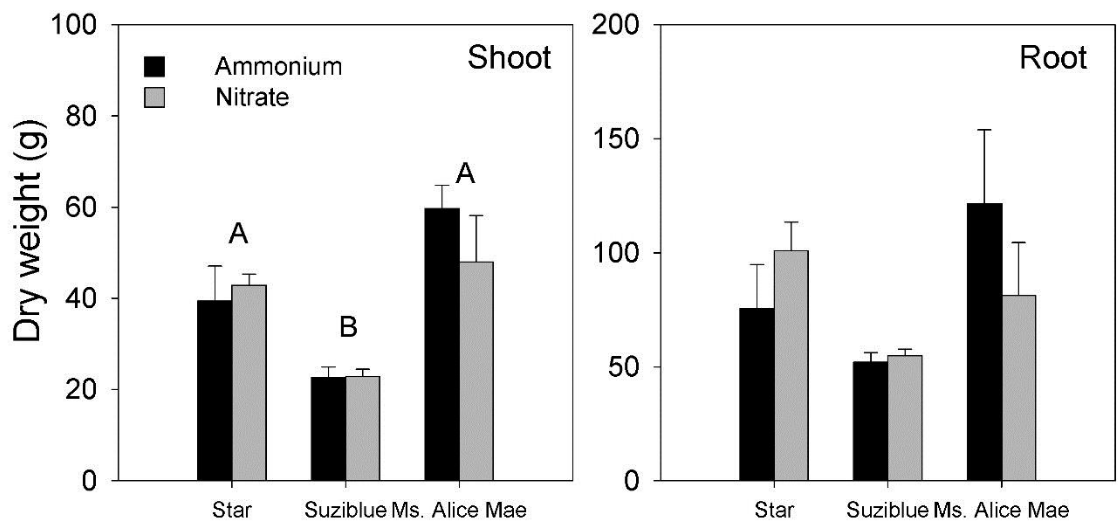

Fig. 3. Dry weight of the shoots and roots in three southern highbush cultivars in response to ammonium and nitrate as sources of nitrogen. Weights were measured at the end of the experiment. Error bars represent the standard error of the mean (n = 4). Similar letters above the bars indicate that the cultivars were not significantly different ($\alpha = 0.05$).

The southern highbush blueberry plants removed up to 16 µmol of nitrogen in the form of ammonium per gram of root dry weight per day (Fig. 4). Within a given cultivar, the rate of nitrogen removal from the nutrient solution was generally similar over the duration of the experiment. ‘Suziblue’ displayed slightly higher rates of ammonium acquisition from the nutrient solution over the duration of the experiment although this was significant ($P = 0.04$) only at 18 d after treatment (Fig. 4). At this stage the extent of ammonium-N uptake in ‘Suziblue’ was 70% higher than that in ‘Ms. Alice Mae’ but not significantly different from that of Star. These data suggest that there is variation in the extent of ammonium-N acquisition capacity among southern highbush blueberry cultivars. It may be likely that at least part of the differences in the rate of ammonium-N uptake are associated with differences in root mass. ‘Suziblue’ tended to display lower root dry weight at the end of the experiment, although this was not statistically significant ($P = 0.051$). Nutrient acquisition including that of N occurs primarily through the terminal and young parts of the root system. It may be likely that the extent of such young roots was generally similar across the three cultivars.

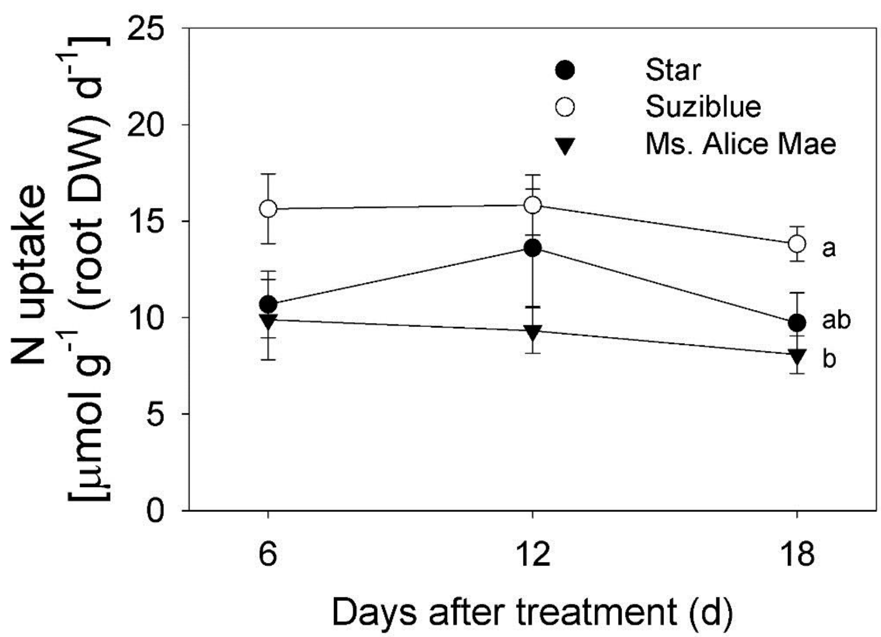

Fig. 4. Nitrogen (ammonium) uptake rates in three southern highbush cultivars in response to ammonium and nitrate as sources of nitrogen. The nitrogen remaining in the hydroponics solution was measured and the N uptake rate was determined after accounting for evapo-transpiration related water loss from container. Error bars represent the standard error of the mean (n = 4). Similar letters next to lines indicate that the cultivars were not significantly different ($\alpha = 0.05$) at the given time point (18 d after treatment).

Nitrate acquisition is currently being determined by measuring the nitrate content remaining in the container. Root and shoot N content measurements as well as enzyme activity measurements are under progress. Samples for these analyses have been collected from the study. These data will provide further information on the N acquisition and metabolism characteristics across the southern highbush blueberry cultivars.

Over the duration of our hydroponics experiments, we have noticed that young (around one year old) plants are best suited for this system. Such plants were not readily available for the rabbiteye blueberry genotypes that we planned to study. Hence, this study has not yet been performed on the rabbiteye blueberry plants. These rabbiteye plants will be obtained in late winter and the study will be performed during early spring of 2018. This will allow for comparisons of N acquisition and metabolism characteristics across different types of blueberry.

Potential Impact

This study will help evaluate potential preferences for ammonium and nitrate forms of nitrogen among some of the important emerging southern highbush and rabbiteye blueberry varieties. This information can be useful in refining and optimizing nitrogen management strategies for southeastern blueberry production. Preliminary analysis suggests that there are potential differences in N acquisition rates among southern highbush blueberry cultivars. If this study indicates potential differences among southern highbush and rabbiteye varieties in terms of their nitrogen acquisition and/or utilization capacities, such information can be directly used to develop type-specific nitrogen applications. Such applications of these data under field production can help in the better establishment of the plants, improve overall productivity and grower profitability.

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