Progress Report – 2022 R-13

Title: Interaction between pH and form of Nitrogen (N) on blueberry growth and N uptake

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Objectives: The main objective of the proposed research is:
To determine the interaction between pH and N-source on blueberry growth and N uptake

Justification and Description:
Blueberry (*Vaccinium* species) is a major fruit crop in the southeastern US and is the leading, cultivated fleshy fruit species in Georgia. Two main types of blueberry are grown in GA, rabbiteye blueberry and southern highbush blueberry. Blueberry cultivation exceeded 26,000 acres and was valued at over $220 million (2019 Georgia Farm Gate Value Report). Continued profitability of blueberry production requires optimization of various factors currently limiting its production in GA and in other parts of the southeastern United States. Plant nutrition is one such key area where considerable improvements still need to be made to optimize and ensure sustainable production. Applying the right form of nutrients such as N, under the right pH conditions is essential to optimize their availability and plant uptake. Information to make such decisions is currently lacking as much of the current fertilization recommendations are based on older studies or were performed in northern highbush blueberry genotypes.

Blueberry plants display two specific soil and nutrition related requirements: 1. an acidic soil/media pH for optimal growth (Korcak, 1988); and 2. a potential preference for the ammonium (NH_4^+) form over the nitrate (NO_3^-) form of supplied N (Townsend, 1969; Claussen and Lenz, 1999; Poonnachit and Darnell, 2004; Alt et al., 2017; Doyle et al., 2021). Soil pH greatly affects the availability of nutrients to plants and therefore has a profound influence on their cultivation. *Vaccinium* species, including the cultivated blueberry are generally thought to be better adapted to soils with acidic pH, generally between 4 and 5.5 (Cain, 1952). As typical agronomic soils often display relatively higher pH, substantial soil amendments such as application of elemental sulfur prior to planting are needed to obtain the optimum pH for blueberry cultivation. Further, to ensure maintenance of the required low pH, fertilization is often performed using acidifying fertilizers such as sulfates.

Another consequence of their nutritional requirements is that the N source that is typically supplied to blueberry plants is the NH_4^+ form of inorganic N. Although plants can acquire N from the soil in the organic and inorganic forms, the major form in which it is acquired under cultivation

conditions is in the inorganic form supplied through fertilizers. Nitrate and ammonium are the two inorganic N forms available to plants. In many plants overall growth or performance is reduced when they are supplied with NH_4^+ as the only source of N. This has been attributed to a variety of factors including toxicity, ionic imbalance, and energetics of futile cycling of NH_4^+ (Britto and Kronzucker, 2002). However, plants such as blueberry are thought to display preference for the NH_4^+ form of N over the NO_3^- form (Cain, 1952; Townsend 1969; Poonnachit and Darnell, 2004). Multiple studies in blueberry have suggested greater growth and/or higher tissue N content under conditions of NH_4^+ supply (Cain, 1952; Townsend, 1969; summarized in Doyle et al., 2021). The basis for such a potential preference for the NH_4^+ form of N in blueberry is not well understood. It is likely that N uptake from the soil/media, its transport within the plant, and its assimilation play important roles in determining such preference (Poonnachit and Darnell, 2004; Alt et al., 2017). However, some other studies suggest no specific preference and indicate that both forms of inorganic N can be used by blueberry (Oertli, 1963; summarized in Doyle et al., 2021).

The two specific requirements described above for blueberry cultivation may be related. Availability of the NH_4^+ form of N is thought to be relatively higher than that of NO_3^- under acidic pH conditions. Under acidic pH conditions, activity of nitrifying microorganisms such as *Nitrosomonas* sp. and *Nitrobacter* sp. which convert the NH_4^+ form of N to the NO_3^- form, is thought to be reduced, thereby reducing availability of the NO_3^- and allowing for higher NH_4^+ availability. However, recent studies suggest that other microorganisms such as archaea are active under lower pH conditions and are significant players in converting NH_4^+ to NO_3^- under field conditions (Hu et al., 2014). Hence, it remains possible that some nitrification can occur under acidic pH allowing for NO_3^- availability under low pH conditions.

The interaction between pH and the form of N supplied has not been sufficiently well investigated in blueberry. This is particularly true for current cultivars in southeastern blueberry production. Multiple questions regarding N nutrition and its interaction with pH remain unclear. Does NH_4^+ uptake by blueberry plants change with changes in pH? Is NO_3^- uptake affected by media pH? Does presence of NO_3^- influence NH_4^+ uptake when both N forms are available for acquisition? In some plants, synergistic N-uptake and plant growth responses to availability of both forms of inorganic N have been reported (Britto and Kronzucker, 2002). It is unclear if this occurs in blueberry and if such effects are influenced by media pH. Finally, is the effect of pH on blueberry growth mediated in part by nutrient acquisition characteristics? Such information is essential for making decisions on specific recommendations for N nutrition and optimum pH for blueberry cultivation as N forms can typically be present together in cultivated soils, and as pH may vary across cultivation sites. Hence the objective of the work proposed here is to determine the interaction between media pH and form of N supplied on blueberry plant growth and N uptake.

Materials and Methods

Plant material

The southern highbush cultivar, ‘Suziblue’, was utilized for this trial. Plants were purchased as cuttings from Alma Nursery and Blueberry Farms in Alma, GA. Plants were transplanted into 1.6 L containers in a 1:1 peat moss: pine bark medium. Plants were grown for around two years under greenhouse conditions. Plants were fertilized at 50 ppm N with J.R. Peter’s Acid Special fertilizer following Georgia recommendations for blueberry fertilization. Prior to acclimation in the hydroponic system, plants were not watered or fertilized for 5-d to ensure uniformity among plants.

Hydroponic System

The hydroponic system utilized for this trial consisted of 54, 0.95 L containers purchased from a local hardware store. Three air pumps were used to supply air via airline tubing to each of the containers. Plants were suspended from a trellis system in the greenhouse where the rootzone collar was suspended around 2.5 cm above the hydroponic solution. The hydroponic solution was modified from Hoagland's hydroponic solution for use with blueberry: 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, and 1 millimole of N supplied either as potassium nitrate, ammonium sulfate or ammonium nitrate. The acclimation and treatment solutions supplied 1 mM N, whereas the starvation solution consisted of the modified Hoagland's solution without N. The time frame for the experiment was 5 d for acclimation, 3 d for starvation and 28 d for the treatment solution. During treatments, the hydroponic solution was changed every 7 d and pH adjusted manually using 1 M hydrochloric acid (HCl) or potassium hydroxide (KOH) every 2- to 3-d. The treatments consisted of two factors: 1. N-source with three levels, NH_4^+, NH_4NO_3 and NO_3^-; and 2. pH with three levels: 4.0, 5.0, and 6.0. The experiment was set up as a randomized complete block design with six replicates. Each plant was treated as an experimental unit.

Data collection

Prior to each adjustment of pH (performed at 2 to 3-d intervals), the pH of the nutrient solution was measured using a portable pH meter. Chlorophyll content index measurements were collected at 7-d intervals following the initiation of the experiment. Chlorophyll fluorescence measurements were collected using a FluorPen FP100. Quantum yield data were collected using this instrument. Data were collected under ambient light conditions and following dark adaptation of leaves for 30 min using an aluminum foil. The dark-adapted chlorophyll fluorescence data are equivalent to the maximum quantum yield of PSII. Chlorophyll fluorescence data were collected at three time points (7-d intervals). Chlorophyll content index and dark-adapted chlorophyll fluorescence data are shown for the final time point. Shoot and root samples were collected to evaluate biomass accumulation and elemental analysis of roots and shoots to determine if N-source influences these aspects. Fresh weight data were recorded at harvest. Dry weight data were collected following drying at 65 °C for 5 d. Elemental analysis (*in progress*) will be conducted at Waters Environmental Laboratories in Albany, GA. The samples for elemental analysis will be oven dried at 65 ºC for 5 d, then finely ground using a ball mill and submitted following Waters Environmental Laboratories guidelines for sample submission. During week 3 of the experiment, nutrient solution was collected at 2 d after refreshing the nutrient solution. Amount of solution remaining in the containers were also recorded at this stage. The samples will be used to determine the NH_4^+ and NO_3^- concentrations. These will be used to subsequently determine the total N uptake in response to the N-source and pH treatments.

Statistical analyses

Statistical analyses were performed in JMP 15. The block was treated as a random effect. When interactions between factors were significant, test of effect slices was used. Further mean separation was performed using Tukey's HSD. When main effects were significant, means separation was performed using Tukey's HSD. Figures were prepared in SigmaPlot 14.

Results and Discussion

The pH of the nutrient solution was adjusted at 2-3 d intervals. Prior to the adjustment, the pH of the solution was recorded. At 7 d after initiation of the treatments, the main factor, pH (target) affected the measured pH as may be expected (Fig. 1). However, at this stage, the N-source also affected the measured pH. Overall, the NH_4^+-N, and NH_4NO_3-N treatments resulted in lower pH than the NO_3^--N treatment. Specifically, presence of NH_4^+ resulted in a decrease in pH of the nutrient solution. At later stages (19 d after treatment is shown as an example), an interaction effect between the two factors was significant. Mean separation analyses indicated that at pH 4.0, the NO_3^--N treatment resulted in higher measured pH (4.27) than the NH_4NO_3-N and the NH_4^+-N treatments (3.63 and 3.45, respectively). At target pH of 5.0, the NO_3^--N treatment resulted in higher measured pH (5.51) than the target and the other two treatments, and the NH_4NO_3-N treatment resulted in higher pH (4.11) than in the NH_4^+-N treatment (3.45). At a target pH of 6.0, NO_3^--N treatment was not substantially different from the target (6.0) but the NH_4^+-N and NH_4NO_3-N treatments were substantially lower (4.27 and 5.14, respectively). Further, the NH_4^+-N treatment was significantly different from that of NO_3^--N treatment. These data suggest that N-source affects the pH of the rhizosphere. While NH_4^+-N tended to decrease the measured pH, NO_3^--N resulted in an increase (or maintenance) in pH. The data presented here are consistent with previous analyses of N-source effects on rhizosphere pH in blueberry (Imler et al., 2019). In the previous study, NH_4^+ uptake was reported to result in root zone acidification while NO_3^- uptake resulted in alkalinization of the root zone. At all pH levels, presence of NH_4^+ in the nutrient solution resulted in substantial further acidification of the rhizosphere. Presence of NO_3^- resulted in rhizosphere alkalinization when the external pH was lesser than 6.0. Uptake of NH_4^+ is often associated with counter transport of H^+ to correct the charge imbalance (Britto and Kronzucker, 2002). Conversely, efflux of OH^- ions to counter the anion influx with NO_3^- supply and the H^+ symport mechanism involved in its uptake may contribute to the alkalinization of the rhizosphere. Analyses of uptake rates of N under the above pH and N-source conditions is likely to provide more insights into the relationship. Such analyses using samples from this study is currently in progress as part of this project.

Availability and utilization of N in plants is closely associated with photosynthetic efficiency. Chlorophyll content index measurements and chlorophyll fluorescence measurements were performed to estimate the impact of pH and N-source on these parameters (Fig. 2). Chlorophyll content index was not significantly altered by either factor during the experiment (data for the final time-point only are presented here). Further, measurement of the maximum quantum yield of PSII indicated no significant differences in response to the pH and N-source factors (data for the final time-point only are presented). Additionally, quantum yield measurements of light adapted leaves yielded similar results. These data suggest that N-source and pH did not influence chlorophyll content of fluorescence parameters. Whether photosynthetic capacity was directly affected remains to be determined. It may be noted that the maximum quantum yield of PSII values were substantially low. Potted plants measured around the same time as the study displayed values closer to 0.8 indicating healthy leaves. The low values (around 0.6) suggest that the plants in hydroponics were likely stressed. It may be speculated that hydroponic culture may have resulted in the additional stress. Analyses of N concentration in the leaves (work in progress) may allow for further insights into the above relationships.

N-source did not significantly affect plant total fresh weight or dry weight (Fig. 3). However, pH influenced total fresh weight. Highest fresh weight was noted at pH 5.0 which was

significantly greater than that at pH 4.0. Analyses of the constituent shoot and root fresh weights indicated that root fresh weight was significantly greater at pH 5.0 compared to that at pH 4.0. These data suggest more optimal growth at pH 5.0. However, it should be noted that although a similar trend was noted with dry weight, it was not statistically significant. Hence, within the short time-frame of the experiment, N-source had no significant effect on plant growth while pH influenced plant fresh weight but not its dry weight.

The project is currently under progress. The following parameters will be determined using samples collected from this study. 1. Tissue analyses: this will be performed to determine the shoot and root nutrient concentrations. 2. N-uptake analyses: samples have been collected to determine NO_3^- and NH_4^+ concentrations at 2 d after replenishing nutrient solutions. These data will help determine the effects of N-source and pH on N-acquisition in the southern highbush blueberry plants.

Figures

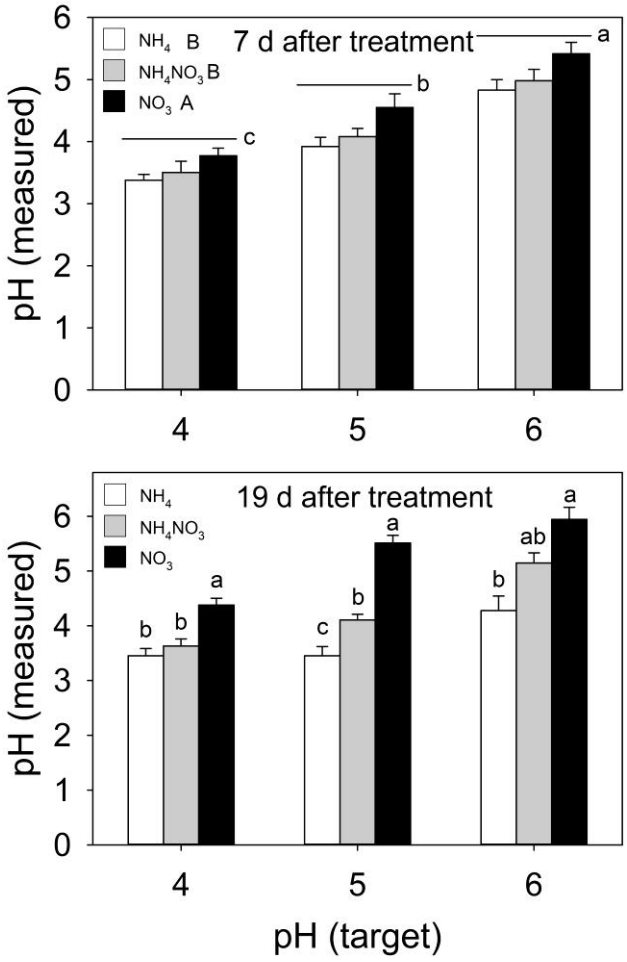

Fig. 1. Changes in nutrient solution pH in response to N-source and target pH in 'Suziblue' blueberry. The pH of the nutrient solution was measured at 2 d (7 d after treatment) or at 3d (19 d after treatment) after pH was adjusted to the target level. Capital letters next to the labels in the upper panel indicate mean separation results within the N-source treatment. Small letters in upper panel indicate mean separation results in the pH treatment. In the lower panel, interaction effects between N-source and pH were significant. Hence, Tukey's HSD mean separation analyses were performed separately within each pH level. Similar letters indicate that corresponding values are not significantly different.

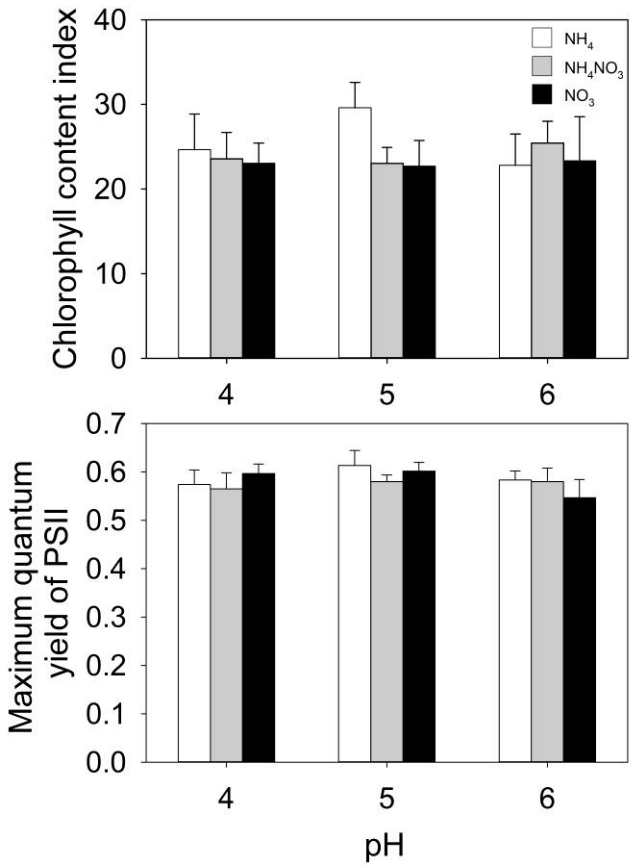

Fig. 2. Chlorophyll content index and chlorophyll fluorescence changes in responses to N-source and pH in 'Suziblue' blueberry. Chlorophyll meter was used to measure the chlorophyll content index. FluorPen FP100 was used to measure quantum yield in dark adapted leaves to obtain the maximum quantum yield of PSII. Data are from week 3 after initiation of treatments.

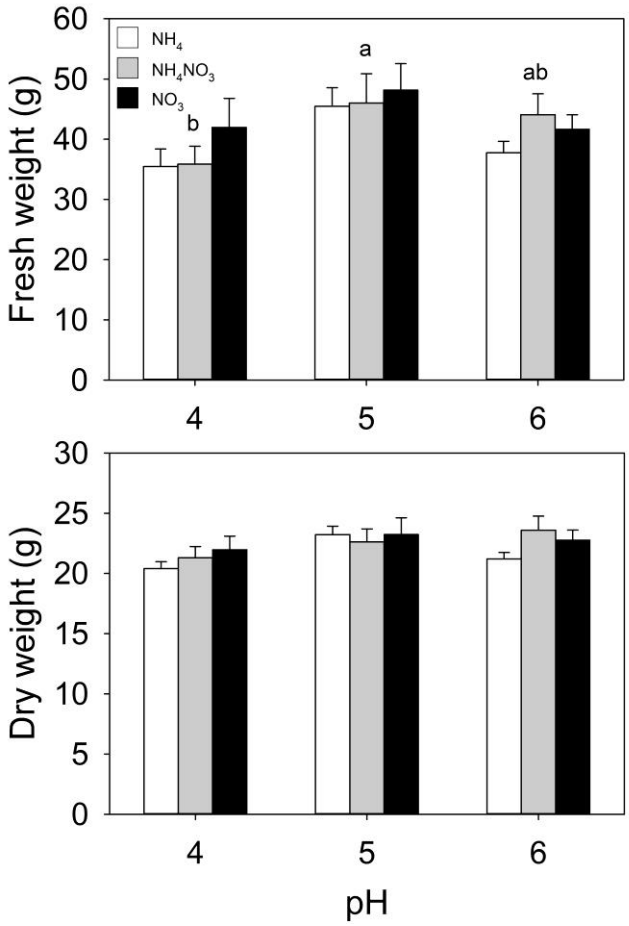

Fig. 3. Total fresh weight and dry weight in response to N-source and pH in 'Suziblue' blueberry. Shoot and root fresh weight and dry weight data were collected at the end of experiment (4 weeks after initiation). Small letters above bars indicate results of mean separation of the pH treatment factor. Similar letters above bars indicate that the corresponding pH treatments are not significantly different.

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