ABSTRACT

MASON II, JAMES HUNTER. Nitrogen Management for Field and Greenhouse Production of Flue-cured Tobacco. (Under the direction of Dr. Matthew Vann).

Nitrogen (N) management is key to the successful production of flue-cured tobacco (*Nicotiana tabacum* L.), both as seedlings and mature plants. Understanding N management, forms, application timings, and rates, can reduce input and might increase yield, quality, and value of tobacco. To evaluate these parameters, three research projects were conducted from 2015 to 2017 to determine the effects of N application programs to organically produced seedlings and conventionally grown field plants.

The first study evaluated three different N rates applied at different growth stages in the Piedmont region of North Carolina. Treatments included base N rates of 56, 78, and 101 kg/ha that were delivered in two or three application timings. Timings included 1) 7 to 10 days after transplanting and at layby; 2) 7 to 10 days after transplanting, at layby, and two weeks after layby. Crop value, SPAD measurements at topping, and N concentration at layby, at topping, and after curing, were not affected by treatments across all environments. The highest crop yield was recorded at the 101 kg N/ha rate, which is likely due to environmental conditions. At one location crop quality decreased as N application timings increased from two to three. In fine-textured soils, two N applications appear to be sufficient and show be delivered by layby to prevent late-season N assimilation.

In the second study, organic seedling fertility studies were evaluated in a float-bed production system. A total of seven treatments were evaluated. The objective of this study was to compare a conventional 16-5-16 fertilizer program to organic programs consisting of sodium
nitrate (16-0-0), pelleted Peruvian seabird guano (12-11-2), or a combination of both N sources, and also to quantify nutrient availability and seedling growth. Concentrations of ammonium, nitrate, and inorganic N, along with bicarbonate, pH and dissolved oxygen were quantified in the solution of each float bed every five days throughout the study. In addition, total and usable plants produced were quantified at the conclusion of the study, along with stem height and diameter. All treatments were compared against each other to potentially provide an effective fertility program for organic tobacco transplant production. Results from both environments show greater bicarbonate and ammonium concentration in the float water solution where seabird guano was applied as the exclusive source of N. An increase in nitrate was observed as ammonium concentration decreased, suggesting nitrification rates increased as the seabird guano was exposed prolonged environmental conditions. Five of seven treatments produced an acceptable percentage of usable plants. Two treatments which did not produce usable plants did not contain phosphorus as a portion of the fertility program.

The third study evaluated the impact of float bed aeration to nutrient release and availability, total and usable plants produced, and stem height and diameter among five fertility treatments used for organic tobacco transplant production. Four of the treatments were comprised of organic N sources (Peruvian seabird guano, sodium nitrate, or a combination of both), while a conventional 16-5-16 fertilizer was used as a control treatment. Each of the five treatments were placed in non-aerated and aerated float beds. Aeration was provided by a Hydor bubble maker. Additional data collected throughout this study included dissolved oxygen concentration of the float bed solution, water temperature, pH and inorganic N. Results from this experiment demonstrate a reduction of bicarbonate and ammonium concentration, coupled with an increase in nitrate in aerated beds.
Nitrogen Management for Field and Greenhouse Production of Flue-cured Tobacco

by

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DEDICATION

This thesis is dedicated in memory of my grandparents, John and Mona Pass and Wallace Mason. I also dedicate this thesis to my parents, Bruce and Johnsie Mason who have sacrificed so much to provide our family a wealth of opportunities, many of which I will never be able to repay them for. In addition, this thesis is dedicated to my sister, Leigh Mason, who has added wisdom and humor throughout my entire life. Lastly, I would like to dedicate this thesis to my grandmother, Mildred Mason, who continues to encourage me to chase my dreams and never give up hope. To this day, each of these individuals has supported me, encouraged me, taught me right from wrong, and instilled in me the values of hard work, honesty, gratitude, and faith. I would not be where I am today without the help of these people in my life.
BIOGRAPHY

James Hunter Mason II, son of Bruce and Johnsie Mason, was born in Winston-Salem, North Carolina on February 12th, 1993. He was raised in the outskirts of Winston-Salem and graduated from Ronald Reagan High School in 2011.

In 2015, Hunter graduated from North Carolina State University with a Bachelor of Science degree in Agricultural Business Management. Hunter’s passion and interest in agriculture, specifically tobacco production, led him to seek further education. In March of 2015, Hunter was admitted to graduate school at North Carolina State University where he began to pursue a Master’s of Science in Crop Science degree under Dr. Matthew Vann. While in graduate school, Hunter was a member of the Crop and Soil Science Graduate Student Association and served as the Treasurer for the 2016-2017 academic year. Upon graduation, Hunter plans on beginning a career with Universal Leaf Tobacco Company as an agronomist.
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The author would like to express great gratitude to Drs. Matthew Vann and Loren Fisher for giving him the opportunity of a lifetime. The guidance, friendship, support, and learning opportunities they have provided is appreciated more than they can imagine. A sincere appreciation is also expressed to Dr. Michelle McGinnis for her wisdom, knowledge, time and support while serving as a graduate committee member.

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CHAPTER ONE

Late Season Nitrogen Application to Flue-cured Tobacco (*Nicotiana tabacum* L.) Produced in Fine-textured Soils

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ABSTRACT

Late-season nitrogen (N) assimilation can greatly impact the yield and quality of flue-cured tobacco (*Nicotiana tabacum* L.). Previous research efforts focused to coarse-textured soils in the Coastal Plain region of North Carolina indicate that late-season N applications can be successfully utilized to slow senescence and improve yield under certain growing conditions. At present, little is known regarding the implications of late season N application to flue-cured tobacco produced in the fine-textured soils that characterize the Piedmont region of North Carolina. Research was conducted at four on-farm locations from 2015 to 2016 to evaluate the effect of N application rates and the number of applications to the yield, quality, value, and leaf chemistry of flue-cured tobacco (*Nicotiana tabacum* L.). Liquid N (28% Urea-Ammonium-Nitrate) was applied at 56, 78, and 101 kg N/ha. Nitrogen was either split applied at a one-half rate 7-10 Days after Transplanting (DAT) and a one-half rate at layby, or in a one-half rate 7-10 DAT, a one-quarter rate at layby, and a one-quarter rate Two Weeks After Layby (2WAL).

Leaf tissues samples were collected at three different crop stages: at layby (when plant height was approximately 36 cm), at topping (when plant height was roughly 120 cm), and after curing to determine total N concentration of the leaf. In addition, cured leaf tissue samples were also collected to measure total alkaloid and reducing sugar. Plots were harvested and green weight was recorded. Cured leaf was calculated using 85% reduction in weight during the curing process. A subsample of harvested green leaf was cured and assigned a USDA grade to determine quality. Crop value per hectare was determined through combination of yield and quality. Composite cured leaf samples were also collected for leaf chemistry quantification. Data were analyzed in SAS version 9.4 and subjected to analysis of variance (ANOVA) using PROC GLM and treatment means were separated using Fisher’s Protected LSD at p ≤ 0.05.
Results from this study demonstrate an increase in yield as N application rate increased from 56 to 101 kg N/ha; however, crop value was not affected. This suggests that there was likely a decrease in leaf quality as yield increased. A decrease in quality was observed in some environments as N application number increased from two to three. Results indicate that current N application rates and timings are adequate to obtain optimum yield, quality and value on fine-textured soils similar to the Piedmont region of North Carolina.
NOMENCLATURE

Nicotiana tabacum L.

KEY WORDS

Nitrogen, rate, application timing

INTRODUCTION

N Forms and Plant Uptake

Inorganic N in soil is primarily derived from three sources: soil organic matter, atmospheric N, and N fertilizer (Franco and Munns, 1982). The atmosphere provides a vast reservoir of molecular N (N₂). When atmospheric N₂ is fixed, the first form of combined N is ammonium (NH₄⁺), and most of it is immediately assimilated into organic forms so that very little is exuded to soil (Franco and Munns, 1982). This form of N is not available for use by plants and must first be converted to a fixed form either by oxidation to nitrate (NO₃⁻) or by reduction to NH₄⁺. During decomposition of organic matter in most agricultural soils, excess NH₄⁺ not utilized by microbes is released, and subsequently oxidized by autotrophic bacteria to nitrite (NO₂⁻) and then NO₃⁻ (Franco and Munns, 1982). The N of NO₃⁻ is assimilated in the process of reduction and subsequent amination, while NH₄⁺ assimilation also involves an amination process (Mengel and Kirkby, 2001).

Various forms of N may be absorbed by plants; however, NO₃⁻, NH₄⁺, N₂ account for most of the N used in natural ecosystems and in agriculture (Franco and Munns, 1982). Three major pathways facilitate N absorption by the plant. These pathways include root contact (roots
grow in the direction of favorable conditions where the favorable condition/nutrient is located), diffusion (nutrient movement with soil water from an area of high concentration to an area of lower concentration), and mass-flow (nutrient movement with soil water movement) (Tisdale and Nelson, 1975). When supply by mass flow is restricted, such as when moisture is limiting, diffusion becomes a major mechanism of nutrient supply (Halstead et. al., 1968; Brewster and Tinker, 1970; Bole and Barber, 1971; Elgawhary et al., 1972). However, mass flow may be a dominant process for non-adsorbed nutrients with high solubilities, such as NO\textsubscript{3}\textsuperscript{-} (Davidson et al., 1978). In contrast to other plant nutrients, N may be assimilated as a cation NH\textsubscript{4}\textsuperscript{+} or an anion NO\textsubscript{3}\textsuperscript{-} (Mengel and Kirkby, 2001). Both ions move to the root surface through mass flow and diffusion or are intercepted through root contact (Havlin et. al., 1999). Uptake rates are determined mainly by the physiological need of the plant and not so much on whether the source is a cation or anion (Mengel et al., 1983). However, McCants and Woltz (1967) have stated that NO\textsubscript{3}\textsuperscript{-} is the preferred form of N for high quality tobacco, relying on results from greenhouse and field experiments.

Nitrate is absorbed by plant roots and is either reduced, stored in the vacuoles, or translocated to the shoot for reduction and vacuolar storage (Tischner, 2000). Evidence indicates that NO\textsubscript{3}\textsuperscript{-} uptake is dependent upon photosynthesis and carbohydrate supplied from the shoot (Ciobotari et al., 2009). The process initiated by the exposure of plants to NO\textsubscript{3}\textsuperscript{-}, which leads to a continuous increase of NO\textsubscript{3}\textsuperscript{-} uptake, is termed induction (Tischner, 2000). This process includes an increase in the concentration of NO\textsubscript{3}\textsuperscript{-} carriers in the plasma membrane. The uptake into the root symplast from the outer medium depends on the concentration outside the root, whereas the xylem loading process depends on the shoot demand and the content of reduced N compounds in the phloem (Saravitz et al. 1998).
There are many notable differences between the assimilation of NH$_4^+$ and NO$_3^-$. The uptake rate of NO$_3^-$ is dependent on the energy status of plants (Rufty et. al. 1989). When the plant is going through much higher rates of photosynthesis due to interception of sunlight the plant synthesizes more carbohydrates, which can lead to an increase in the uptake of nitrates (Rufty et. al., 1989). An important factor that dictates the uptake of ammonium and nitrate N is pH. According to Mengel and Kirkby (2001), ammonium assimilation occurs best in a neutral medium and is depressed as the pH is reduced; however, a more rapid uptake of nitrate occurs at low pH values because more protons are available for the proton cotransport of nitrate. At low pH levels in the nutrient solution, as brought about by NH$_4^+$, root growth is often depressed (Mengel and Kirkby, 2001). According to Oscarson and Larsson (1986), the net uptake of NO$_3^-$ results from nitrate influx and efflux. In contrast to NH$_4^+$, nitrate can be transported at high rates into upper plant parts and can be stored at high concentrations in the vacuole (McIntyre, 1997).

While the form of N used to supply a tobacco crop has been disputed, recent experiments conducted in North Carolina have consistently shown that programs utilizing all-nitrate or majority ammonium N products produce tobacco leaf with similar yield and quality (Vann and Inman, 2016). The use of Urea-Ammonium-Nitrate (UAN) as a portion of the entire amount of required N has become a popular practice (Vann and Smith, 2014). Parker (2009) reported that N source did not affect yield, quality index, dollars per hectare, total alkaloids, total reducing sugars, or leaf color. In addition, UAN may also save growers as much as 40 to 45 percent on fertilizer cost (Vann and Smith, 2014), when compared to other N sources.
Assimilation

Nutrient assimilation generally requires large amounts of energy to convert stable, low-energy inorganic compounds into high-energy organic compounds (Taiz and Zeiger, 2010). For example, the reduction of nitrate to nitrite and then to ammonium requires the transfer of about 10 electrons and accounts for about 25 percent of the total energy expenditures in both roots and shoots (Bloom, 1997). While N is plant available in the inorganic form, both nitrate and ammonium must be assimilated back into an organic form before it can become part of the building blocks of amino acids and amides (Haynes, 1986).

Plant roots actively absorb nitrate from the soil solution via several low- and high-affinity nitrate-proton cotransporters (Crawford and Forde, 2002; Miller et al., 2007). Plants eventually assimilate most of this nitrate into organic N compounds (Taiz and Zeiger, 2010). The first step of this process is performed in the cytosol by nitrate reductase (Tischner, 2000). Nitrate reduction occurs in two steps, the first mediated by nitrate reductase and the second by nitrite reductase, both of which are nitrate inducible (Breteler et al., 1979; Jackson, 1978). The most common form of nitrate reductase uses only NADH as an electron donor (Taiz and Zeiger, 2010); however, another form of the enzyme that is found predominantly in non-green tissues such as roots can use either NADH or NADPH (Warner and Kleinhofs, 1992). Nitrate reductase of higher plants is composed of two identical subunits, each containing three prosthetic groups: flavine adenine dinucleotide (FAD), heme, and a molybdenum atom complexed to an organic molecule called perin (Campbell, 1999). After the FAD-binding domain accepts two electrons from NADH or NADPH, these same electrons then pass through the heme domain to the molybdenum complex, where they are transferred to nitrate (Taiz and Zeiger, 2010). It should be noted that molybdenum is required as a cofactor and that the NADH/NADPH is sourced from
respiration in the roots along with photosynthesis in the leaves, depending on where the reduction takes place (Barker and Bryson, 2007). As opposed to ammonium, plants can store high levels of nitrate; or translocate it from tissue to tissue without deleterious effect (Taiz and Zeiger, 2010). Nitrate can be stored in vacuoles in the roots, translocated in the xylem and stored in vacuoles of leaf tissue, or reduced by nitrate/nitrate reductase and stored in the leaf tissue (Engels and Marschner, 1995; Barker and Bryson, 2007).

Assimilation of NH$_4^+$ in the roots requires carbohydrates translocated from shoots to roots provide the carbon skeletons and the energy (ATP and NADPH) for the NH$_4^+$ assimilation process (Mengel and Kirkby, 2001). Most of the NH$_4^+$ taken up by the roots is assimilated in the roots and translocated in the form of amino acids and amides to the shoots (Mengel and Kirkby, 2001). Ammonium, if accumulated at high levels in living tissues, can be toxic to plants (Taiz and Zeiger, 2010). Plant cells avoid ammonium toxicity by rapidly converting the ammonium generated from nitrate assimilation or photorespiration into amino acids (Taiz and Zeiger, 2010). The primary pathway for this conversion involves the sequential actions of glutamine synthase and glutamate synthase (Lea et al., 1992). Glutamine synthase (GS) combines ammonium with glutamate to form glutamine (Taiz and Zeiger, 2010). Plants contain two classes of GS, one in the cytosol and the other in the root plastids or shoot chloroplasts (Taiz and Zeiger, 2010). The cytosolic forms are expressed in germinating seeds or in the vascular bundles of roots and shoots and produce glutamine for intracellular N transport (Taiz and Zeiger, 2010). The GS in root plastids generates amide N for local consumption; the GS in shoot chloroplasts reassimilates photorespiratory NH$_4^+$ (Lam et al., 1996). Elevated plastid levels of GS stimulate the activity of glutamate synthase (GOGAT) (Taiz and Zeiger, 2010). There are two types of GOGAT found in plants, one accepts electrons from NADH and the other accepts electrons from ferredoxin (Taiz
and Zeiger, 2010). In roots, NADH-GOGAT is involved in the assimilation of NH$_4^+$ absorbed from the rhizosphere, while in vascular bundles of developing leaves, NADH-GOGAT assimilates glutamine translocated from roots or senescing leaves (Taiz and Zeiger, 2010).

**Nitrogen Transport within the Plant**

Nitrogen translocation is one of the most important aspects of plant life. In considering control of N transport into the root, one must be aware of regulation of translocation out of the root symplasm across the xylem into the apoplast of the steele, where molecules move through pits in the walls of the mature xylem vessels and are translocated to the shoot in the transpiration stream (Lauchli, 1976). While translocation into the xylem is regulated separately from uptake into the symplasm, it is also energy dependent and in most cases more sensitive to metabolic disruption than uptake (de Boer et. al., 1983). When the supply of N from the root medium is inadequate, N from older leaves is mobilized to feed the younger plant organs (Mengel and Kirkby, 2001). The previous statement explains why N deficiencies are first experienced on larger, more mature tobacco leaves further down the stalk. N absorbed by plant roots is translocated in the xylem to the upper plant parts (Mengel and Kirkby 2001). During the vegetative stage, the leaves are a sink for N; however, during senescence this N is remobilized for reuse in the developing seeds, mainly amino acids (Okumoto and Pilot, 2011).

Two forms of N that are plant available, NO$_3^-$ and NH$_4^+$, are transported throughout plants in different ways. The form in which N translocation occurs depends on the N uptake source and root metabolism (Mengel and Kirkby, 2001). Ammonium taken up by the roots is almost completely assimilated in the root tissue and the N is translocated to a great extent in the form of amino acids to the upper plant parts (Mengel and Kirkby, 2001). Nitrate N can be translocated unaltered from the roots to shoots and leaves but this depends on the nitrate
reduction potential of the root. Nitrate and amino acids are thus the main form in which N is translocated in the vascular system of higher plants (Mengel and Kirkby, 2001).

**Function and Role of N in Flue-Cured Tobacco**

Among the elements essential for the commercial production of tobacco, none has a more pronounced effect or requires the degree of attention in fertility practices as N (McCants and Woltz, 1967). From the seedling stage through final harvest, the soil N regime affects the process of plant development more than any other mineral element, as appropriate levels are needed to produce a high yielding, quality leaf, with appropriate aroma and smoke flavor (McCants and Woltz, 1967). Soil N must be sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly ensuring a quality leaf (Elliot, 1970; Felipe and Long, 1988; Maw et al., 1995). In general, it is recommended that growers apply between 56 and 90kg N/ha depending upon soil type and cropping history (Vann and Inman, 2016). The lower portion of the range is suggested for fine-textured, fertile soils, while the higher portion of the range is suggested for coarse-textured soils with topsoils deeper than 38 cm to the subsoil (Vann and Inman, 2016).

Water supply is one of the major factors affecting N utilization by tobacco plants (Tso, 1990). Tobacco needs a liberal, well distributed rainfall to provide an adequate water supply for rapid root growth and plant growth and attainment of large leaf size (Tso, 1990). During the early establishment of a tobacco plant, growth is slow and reserves of available soil water should be sufficient to support the plant through the first six weeks (Maw et al., 2009). Maw et al. (2009) states that an imposition of drought from 14 to 30 days after transplanting is beneficial in
stimulating root development. However, in later stages of rapid growth, the amount of available water through rainfall or irrigation may permanently affect development of the plant, either in yield or leaf quality (Maw et al., 2009). According to Maw et al. (2009), tobacco plants benefit from having water available when needed, but water in excess of plant requirements is not recommended. Slight excesses in rainfall produces a rapid, large growth of leaf which when cured is light in weight, thin in body, pale in color, less gummy, and reduced in nicotine content and in aroma (Tso, 1990). As little as 16 kg N/ha above recommendation may reduce leaf quality (Vann and Inman, 2016). Excessive N delays flowering and ripening by prolonging the vegetative state through extended dominance of protein metabolism (Tso, 1990). This delay in maturity, which results from excessive N, prevents normal development of the leaves, and thus when they are cured they lack the desired chemical and physical properties (McCants and Woltz, 1967). The normal ripening process is caused by partial N starvation, which should begin around floral initiation (Vann and Inman, 2016). Prolonged drought can extend N uptake beyond flowering and therefore delay ripening (Vann and Inman, 2016).

Applying the proper quantity of N is just one factor that can impact growth, yield, quality, and value of flue-cured tobacco. The timing of N application is also an important factor affecting growth and development (Tso, 1990). The uptake of N during the first three weeks is quite small, whereas the uptake during the fourth to seventh week after transplanting is generally quite high (Collins and Hawks, 2013). In general, half of a growers target rate of N is applied 7-10 days after transplanting while the remaining half is applied at layby. However, depending on environmental conditions experienced in a particular growing season, it is possible that growers may be able to distribute N applications in more than two intervals.
Justification of Research

Tobacco has played a vital role in the United States since Jamestown colonists arrived in 1612. Tobacco is the most important economic crop which grew up with colonial America and also helped America to grow as a country (Tso, 1990). It continues to be a prominent source of income for families in the United States. In 2015, it was estimated that the United States produced 210.7 million kilograms of flue-cured tobacco on roughly 87,320 hectares (Brown and Snell, 2016).

As previously described, N is a key nutrient to the successful growth and development of tobacco. However, further research is needed to ensure that N is used efficiently. While current recommendations are that N be applied at two intervals, research is needed to determine whether increasing the number of applications could be useful for growers to develop a higher quality and higher yielding crop. Furthermore, applying N in more than two intervals might also delay senescence if curing space is limited. Under current practices, the majority of growers will sidedress the first fertilizer application one week after transplanting (Drake, 2013). Liquid fertilizer, such as UAN, is typically sprayed in an opened furrow before it is ultimately incorporated into the soil profile to reduce volatilization and maximize availability for plant uptake. This same practice occurs for the second fertilizer application at the lay-by growth stage of the plant (four to six weeks after transplanting).

Applying N in three applications could result in higher nutrient use efficiency and be cost effective for growers, as opposed to the two application approach. Applying N in a micro-managed method has many potential benefits. If all N is applied early season (prior to four weeks after transplanting), it is possible that excess precipitation might cause N to leach out of the
topsoil, thus making it difficult for root access. Determining the correct amount of N to adjust for leaching losses is one of the most difficult and risky tasks in tobacco production (Collins and Hawks, 2013). In addition, excess rainfall can cause immature leaves to appear ripe even if they are not ripe from a physiological standpoint (Drake, 2013). All of these factors negatively impact the yield and quality of flue-cured tobacco and could potentially be avoided by adopting a multiple application program. Utilizing this application program would allow growers to base their N application on experienced growing conditions specific to each season. In a growing season characterized by minimal rainfall, excess N is commonly found in the soil profile throughout the growing season. If this N is still present late-season, and rainfall increases, it is likely that the N will be absorbed by plant roots and delay ripening. Applying a smaller portion of the N at layby, followed by another portion two weeks after layby could prove to be an effective method to continuously supplying the plant with N throughout the growing season. Alternatively, if minimal rainfall is experienced between the second and third application, then a third application may not be necessary. Ultimately, this application program could possibly reduce input costs and environmental impact, as well as the strain of management decisions in years marked by adverse weather conditions. Research is warranted to evaluate the impact of N application programs to the yield, quality, and leaf chemistry of flue-cured tobacco when applied to fine-textured soils.

MATERIALS AND METHODS

Field Procedures

From 2015 to 2016, research was conducted at four on-farm locations in the Piedmont region of North Carolina to quantify the effects of various N application programs. Treatments
were arranged in a randomized complete block design with a factorial treatment arrangement, with each treatment replicated four times in each environment. Row spacing was 1.4 m wide in each environment. Tobacco was produced in plots that were four rows wide by 13.71 meters long, where the center two rows for each plot were used for data collection.

Nitrogen concentration was quantified at three intervals: layby, after topping and after curing. One leaf was collected from the third or fourth position below the apical meristem from five randomly selected plants within each plot at each interval. Tissue samples were dried, ground to pass through a 20-mesh sieve, and delivered to the North Carolina State University Tobacco Analytical Services Laboratory for analysis.

SPAD measurements were collected two weeks after layby and at topping. The chlorophyll meter Soil Plant Analysis Development (SPAD-502) is a simple and portable diagnostic tool that measures the greenness or the relative chlorophyll concentration of leaves (Kariya et al., 1982; Torres-Netto et al., 2005). The more chlorophyll that is present in a leaf, the greener the leaf will appear and the SPAD-502 meter will record a higher numerical value. The average of 10 SPAD measurements were collected from the center two rows of each plot.

In three of the four environments, plots were harvested by four individual stalk positions. In 2015, the Stokes environment was only harvested by three stalk positions, which aligned with the growers normal harvest practices. Green weight was collected in the field at each harvest and 85% moisture loss calculated to estimate final yield. In addition, a subsample of the harvested leaf was collected into a mesh bag for curing. After curing, each plot was assigned a United States Department of Agriculture (USDA) grade for each stalk position. Each government grade has an associated grade index value which describes the leaf maturity and ripeness (Bowman et
al., 1988). A composite cured leaf sample representing the four harvested stalk positions was used to quantify total alkaloids and reducing sugars.

**Field Conditions**

Field conditions (soil characteristics, transplant date, pH, and base N rates) are presented in Table 1. Monthly and cumulative rainfall are presented in Table 2. Urea-ammonium-nitrate (28% UAN) was used to supply all N applied in this study. Nitrogen was applied at the outlined rates with a CO₂ pressurized backpack containing a single TG-3 or TG-5 nozzle. In addition, 381 kg/ha of potassium magnesium sulfate (K-Mag, 0-0-22) was applied by hand in an opened furrow on the same day as the first UAN application. This rate was determined from previous research which shows that increasing rates of K₂O above 84 kg/ha does not significantly improve yield, quality, value, or any chemical constituents (Vann, 2011). The K-mag also supplied the magnesium (10.8%) and sulfur (22%) needed for proper plant growth (Fisher, 2014).

A total of 6 treatments were evaluated in each environment. Three treatments consisted of three separate application timings: after transplanting (AT), at layby (AL), and two weeks after layby (2WAL) (Table 3). The remaining three treatments followed current application practices where N is split-applied with one-half rate AT and the remaining half-rate AL. Three base rates of N, 56, 78, and 101 kg/ha, were targeted in specific treatments. Each base rate was applied at 100% of the targeted rate with various combinations of application timings (Table 3). Nitrogen was either split applied at a one-half rate 7-10 DAT and a one-half rate AL, or a one-half rate 7-10 DAT, a one-quarter rate AL, and a one-quarter rate 2WAL. For treatments that received a third application of N 2WAL, UAN was applied to the soil surface on the side of the bed and was not incorporated due to the size of the tobacco plants.
Analytical Procedures

Nitrogen. Leaf N concentration was measured at three different growth stages: at layby, after topping, and cured leaf by the North Carolina State University Tobacco Analytical Services Lab. The Macro-Kjeldahl method was used but with modifications as described by Nelson and Sommers (1973), where leaf lamina was ground to 20-mesh material to improve N recovery and shorten measurement time for each sample.

Total Alkaloids and Reducing Sugars. Total alkaloids and reducing sugars were quantified by the North Carolina State University Tobacco Analytical Services Laboratory using a Perkin-Elmer Autosystem XL Gas Chromatograph system. Fifty-gram cured leaf samples were prepared for each plot by compositing cured leaf from each priming on a weighted-mean basis. Oven-dried samples were ground to pass through a 1-mm sieve and analyzed for percent total alkaloids and percent reducing sugars using the method of Davis (1976).

Statistical Analysis. Data for crop yield, quality, value, percent total alkaloids, percent reducing sugars, percent N at layby, percent N at topping, percent N of cured leaf, and SPAD measurements were subjected to an analysis of variance (ANOVA) using the PROC GLM procedure in SAS version 9.4. Treatment means were separated using Fisher’s Protected LSD test at p ≤ 0.05.

RESULTS AND DISCUSSION

The interaction of treatment x environment was not significant for yield, value, SPAD measurements at topping, N concentration at layby, N concentration at topping, N concentration after curing (Table 4), therefore results are pooled across environments. A location x application
number interaction was observed with respect to leaf quality; therefore, each environment was evaluated independently (Table 5). In addition, a N rate x application number interaction was observed for SPAD measurements collected two weeks after layby (Table 6).

**Yield, Quality, and Crop Value**

*Yield.* Leaf yield was affected by N application rate with leaf yield being greatest in treatments receiving 101 kg N/ha (Table 4). From the seedling stage through final harvest, the soil N regime affects the process of plant development more than any other mineral element, as appropriate levels are needed to produce a high yielding crop (McCants and Woltz, 1967). The recommended amount of N to apply to a soil is 56 to 90 kg/ha (Vann and Inman, 2016); however, this is subject to change depending on soil type and environmental conditions experienced throughout the growing season. In general, increasing N rates up to a certain threshold can potentially increase yield. This trend was observed in this study across all four environments (Table 4), as 101 kg N/ha produced the greatest yield. Rainfall events within each environment were highly variable. It is probable that increased N rates provided the greatest opportunity for N assimilation as soil moisture was increased following a rainfall event. This observation might not be present in a growing season characterized by normal rainfall distribution. When N was applied at the 101 kg N/ha rate, it is likely that more N was concentrated in the soil profile and therefore making it more available for plants early in the season. Had soil moisture been more consistent throughout the growing season, greener leaf may have been observed, which would have impacted yield and quality. However, if N remains in the soil profile late in the growing season it is possible that N could be assimilated by the plant. This would result in late-season greening of the leaf, which would negatively impact curability and potentially decrease quality.
Quality. An environment x application number interaction was observed for leaf quality (Table 5). Due to this interaction, all environments were analyzed individually. Application number did not significantly impact leaf quality in three (Yadkin, Stokes, and Rockingham) of the four environments (Table 5). In Alamance County, the three application timing program reduced leaf quality (Table 5). Alamance County received above average rainfalls during the months of September and October, while experiencing below average rainfall totals after the third N application in late July (Table 2). Due to low rainfall throughout August, it is possible that the N applied in late-July remained in the soil profile and was not used by the plant. Once rainfall increased in September, it is possible that the N that still remained in the soil profile was used by the tobacco plant. This late-season uptake could have potentially caused the tobacco to become greener, thus impacting curability once the crop was completely harvested in early-October.

In the remaining three environments, there were no statistical differences between application number (Table 5). It appears the only difference seen may be due to environmental conditions experienced in Alamance County as opposed to application number alone. Current recommendations of two N applications are adequate for tobacco production in the Piedmont growing region of North Carolina (Vann and Smith, 2014).

Crop Value. No difference was observed in crop value across locations due to N application rate (Table 4). While treatments receiving the highest N rate appeared to have higher yields, an increase in value did not occur (Table 4). Although applying 101 kg N/ha resulted in the highest yield, it is possible that the leaf quality associated with these treatments was slightly reduced, though not at a statistically significant level. A lower quality crop, albeit higher
yielding, would negatively impact crop value, which might explain why differences were not observed.

**Chemical Characteristics**

*Total Alkaloids.* There were no differences observed when comparing treatments for total alkaloid percentages of cured leaves (data not shown). Total alkaloid percentages increased as N rate increased from the 56 kg N/ha rate to the 101 kg N/ha rate; however results were not statistically different. It is possible that higher yields in plots receiving 101 kg N/ha led to a cured leaf that was larger in area and therefore, alkaloid concentration was diluted. This dilution effect would result in similar total alkaloid percentages between the three different N rates.

*Leaf N Concentration at Layby.* Nitrogen concentration within the leaf was not affected by N application rate (Table 4). The assimilation of N is generally at its highest during the fourth to seventh week after transplanting (Collins and Hawks, 2013), it is possible that no differences were observed at this interval because a large quantity of N was not required for plant growth. It is also possible that uptake was limited by the environmental conditions.

*Leaf N Concentration at Topping.* Leaf N concentration increased with N application rate (Table 4). Typically, when higher rates of N are applied to a crop there is higher N concentration in the leaf tissue, given that there is sufficient moisture for plant uptake. This trend was observed in this study as N rate increased from 56 kg N/ha to 78 and 101 kg N/ha (Table 4).

*Leaf N Concentration in Cured Leaf.* Nitrogen rate impacted leaf N concentration in cured leaf (Table 4). Nitrogen concentration increased with N application rate. As N rate increased from 56 and 78 kg N/ha to 101 kg N/ha, N concentration increased as well (Table 4).
SPAD Measurements A significant N rate by application number interaction occurred for SPAD measurements collected two weeks after layby (2WAL) (Table 6). In general, a typical response should be observed in leaf greenness when an increase in N rate occurs. However, it is likely that environmental conditions impacted SPAD measurements, as periods of drought or excessive rainfall can influence N assimilation by plants. In addition, it must be noted that not all of the treatments had received the entire quantity of N at the time of sampling. There were treatments that received 100% of the N applied by layby, while other treatments did not receive the full N rate until two weeks later. This could explain differences observed between the 101 kg N/ha two application program versus the three application program. Had 100% of the N been applied to all treatments by layby, a N rate x application number interaction may not have been observed.

There were no interactions observed for SPAD measurements collected at topping, and therefore data were combined across all environments (Table 4). SPAD meters were used in an attempt to quantify greenness of leaf, which is would be indicative of chlorophyll presence. There is often a correlation between an increase in leaf N concentration and SPAD measurement (Drake, 2013); however, this was not observed in this study. While nitrogen concentration at flowering was statistically different between the 56 kg N/ha rate and the 78 and 101 kg N/ha rate, this did not correlate to statistically different SPAD measurements (Table 4).

CONCLUSION

In this study, the highest N rate produced a higher yield, but did not increase value. It is possible that the higher N rate produced a slightly greener leaf at harvest, thus making curing
more difficult and perhaps decreasing the quality. This decrease in quality would balance out the higher yield and negate any differences that could have been observed in crop value.

Total alkaloids and reducing sugar percentages vary from year to year depending on environmental conditions experienced throughout the growing season. There is a direct correlation between N percentage and nicotine percentage. Experiments conducted with flue-cured tobacco showed that the concentration of N in the tissue is positively correlated with nicotine and negatively related to the sugar content of the leaf (Woltz et al., 1948). In dry seasons, total alkaloids (nicotine) should decrease as N rate decreases because water is needed for plant uptake of N from the soil profile. If more rainfall was experienced in 2015, it is possible that there would have been statistical differences observed in total alkaloids and reducing sugars.

Overall, results from research conducted in 2015 and 2016 do not suggest that there is any added benefit of applying N in three applications, as opposed to two, in soil types similar to those evaluated in this study. It is likely that rainfall played a significant role in the outcome of this research. Due to little differences observed, current recommendations of N application timing and rate are adequate to achieve optimum yield and quality in the Piedmont growing region of North Carolina.
LITERATURE CITED


Table 1. Soil series, pH and transplant date at each environment.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Soil Series</th>
<th>Soil pH</th>
<th>Transplanting date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yadkin-15</td>
<td>Clifford sandy clay loam, Fine, kaolinitic, mesic Typic Kanhapludults</td>
<td>6.4</td>
<td>May 12, 2015</td>
</tr>
<tr>
<td>Rockingham-16</td>
<td>Clifford sandy clay loam, Fine, kaolinitic, mesic Typic Kanhapludults</td>
<td>5.2</td>
<td>May 20, 2016</td>
</tr>
<tr>
<td>Alamance-16</td>
<td>Cecil sandy loam, Fine, kaolinitic, thermic Typic Kanhapludults</td>
<td>6.4</td>
<td>May 26, 2016</td>
</tr>
</tbody>
</table>
Table 2. Rainfall by month for each environment<sup>a</sup>.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>--</td>
<td>--</td>
<td>0.00</td>
<td>9.78</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>May</td>
<td>3.50</td>
<td>10.95</td>
<td>4.97</td>
<td>11.89</td>
<td>14.55</td>
<td>9.70</td>
<td>12.65</td>
<td>9.09</td>
</tr>
<tr>
<td>July</td>
<td>10.82</td>
<td>10.41</td>
<td>8.05</td>
<td>12.29</td>
<td>15.16</td>
<td>12.22</td>
<td>13.54</td>
<td>12.32</td>
</tr>
<tr>
<td>August</td>
<td>11.40</td>
<td>8.46</td>
<td>11.28</td>
<td>9.93</td>
<td>11.81</td>
<td>9.96</td>
<td>4.88</td>
<td>10.79</td>
</tr>
<tr>
<td>September</td>
<td>12.27</td>
<td>10.16</td>
<td>28.50</td>
<td>11.33</td>
<td>9.80</td>
<td>10.62</td>
<td>18.49</td>
<td>9.65</td>
</tr>
<tr>
<td>October</td>
<td>8.30</td>
<td>9.37</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>10.08</td>
<td>8.51</td>
</tr>
<tr>
<td>Total</td>
<td>52.33</td>
<td>59.33</td>
<td>61.23</td>
<td>65.05</td>
<td>67.52</td>
<td>52.50</td>
<td>69.04</td>
<td>60.19</td>
</tr>
</tbody>
</table>

<sup>a</sup>Rainfall totals represent transplanting date to final harvest.
Table 3. Nitrogen application timing and total nitrogen quantity applied.

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>N Application Timing</th>
<th>Total N applied</th>
<th>---kg/ha---</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At transplanting</td>
<td>At layby&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 Weeks after layby&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>50%</td>
<td>50%</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>50%</td>
<td>50%</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>50%</td>
<td>50%</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>50%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>5</td>
<td>50%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>6</td>
<td>50%</td>
<td>25%</td>
<td>25%</td>
</tr>
</tbody>
</table>

<sup>a</sup>At layby plant height measured approximately 38 cm

<sup>b</sup>Two weeks after layby plants height measured approximately 50 cm
Table 4. Yield, value, SPAD at topping and nitrogen concentration as influenced by nitrogen application rate\(^a\).

<table>
<thead>
<tr>
<th>Rate (kg N/ha)</th>
<th>Yield (kg/ha)</th>
<th>Value ($/ha)</th>
<th>SPAD At topping</th>
<th>Nitrogen Concentration at layby</th>
<th>Nitrogen Concentration at topping</th>
<th>Nitrogen Concentration cured leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>2,524 b</td>
<td>8,354 a</td>
<td>50.9 a</td>
<td>5.03 a</td>
<td>4.23 b</td>
<td>2.63 b</td>
</tr>
<tr>
<td>78</td>
<td>2,530 b</td>
<td>8,181 a</td>
<td>51.2 a</td>
<td>5.13 a</td>
<td>4.47 a</td>
<td>2.73 b</td>
</tr>
<tr>
<td>101</td>
<td>2,757 a</td>
<td>9,048 a</td>
<td>51.7 a</td>
<td>5.18 a</td>
<td>4.52 a</td>
<td>2.86 a</td>
</tr>
</tbody>
</table>

\(^a\)Treatment means followed by the same letter within a column are not significant at the \(\alpha=0.05\) level. Data are pooled across all environments.
## Table 5. Tobacco quality as influenced by the main effect of nitrogen application number\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Number</th>
<th>Stokes-15</th>
<th>Yadkin-15</th>
<th>Rockingham-16</th>
<th>Alamance-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 applications</td>
<td>71 a</td>
<td>82 a</td>
<td>85 a</td>
<td>66 a</td>
</tr>
<tr>
<td>3 applications</td>
<td>67 a</td>
<td>80 a</td>
<td>83 a</td>
<td>58 b</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter in the same column are not significantly different at the $\alpha=0.05$ level. Data are reported by individual environment and are pooled across the main effect of nitrogen application rate.
Table 6. SPAD measurements two weeks after layby as affected by the interaction of nitrogen application rate and application number\textsuperscript{a}.

<table>
<thead>
<tr>
<th>N Rate</th>
<th>Application Number</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>--kg N/ha--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>2</td>
<td>43.64 b</td>
</tr>
<tr>
<td>56</td>
<td>3</td>
<td>44.53 ab</td>
</tr>
<tr>
<td>78</td>
<td>2</td>
<td>44.06 ab</td>
</tr>
<tr>
<td>78</td>
<td>3</td>
<td>44.24 ab</td>
</tr>
<tr>
<td>101</td>
<td>2</td>
<td>45.04 a</td>
</tr>
<tr>
<td>101</td>
<td>3</td>
<td>43.86 b</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter are not significant at the $\alpha=0.05$ level. Data are pooled across all environments.
Table 7. P values for yield, quality, value, leaf nitrogen at layby (AL), leaf nitrogen at topping (AT), leaf nitrogen of cured leaf (CL), SPAD measurements AT, and SPAD measurements two weeks after layby (2WAL).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Environment (E)</th>
<th>Rep</th>
<th>Rate (R)</th>
<th>App. Number (N)</th>
<th>R x N</th>
<th>E x R</th>
<th>E x N</th>
<th>E x R x N</th>
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</thead>
<tbody>
<tr>
<td>Yield</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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</tr>
<tr>
<td>Value</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Quality (Q)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.033</td>
</tr>
<tr>
<td>Q Stokes-15</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.2910</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Q Yadkin-15</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.5739</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Q Rockingham-16</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.5054</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Q Alamance-16</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.0231</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>% N AL</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>% N AT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>% N CL</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>SPAD AT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>SPAD 2WAL</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.033</td>
<td>ns</td>
<td>ns</td>
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</tr>
</tbody>
</table>
Chapter Two

Evaluation of Organic Fertility Programs for Flue-cured Tobacco (*Nicotiana tabacum*, L.)
Seedling Production

J.H. Mason¹, M.C. Vann², L.R. Fisher³, M.S. McGinnis⁴

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ABSTRACT

Organic flue-cured tobacco (*Nicotiana tabacum* L.) production has experienced significant expansion. Despite this expansion, there is very little information available that outlines organic fertility programs for seedling production. To provide this information, research was conducted at Central Crops Research Station (CCRS) in Clayton, North Carolina to evaluate the effect of organic fertility programs to seedling vigor and float water chemistry.

The objective of this study was to compare a conventional fertilizer source (16-5-16), to organic fertilizer sources, such as Peruvian seabird guano, sodium nitrate (16-0-0), or a combination of both. Data collected throughout the entirety of the transplant production season included solution nutrient samples, media samples, water temperature, dissolved oxygen, stem height, stem diameter, total plants, and usable plants.

Bicarbonate concentration was <1 meq/L in treatments absent of seabird guano, but reached a high of 14 meq/L when seabird guano was the exclusive N source. Despite high ammonium and bicarbonate concentrations, neither factor is believed to have negatively impacted seedling growth. The two treatments which did not produce any usable transplants did not contain any phosphorus in the sodium nitrate based fertility program. Complete greenhouse fertilizer sources are not available for organic tobacco production; therefore, producers should consider the integrated nutrient plans evaluated in this study to reduce the potential for high ammonium and bicarbonate concentration and low phosphorus concentration.
NOMENCLATURE

Nicotiana tabacum L., tobacco

KEY WORDS

nitrogen, organic, fertility

INTRODUCTION

Flue-cured Tobacco Transplant Fertility

Commercial greenhouse production of tobacco transplants first appeared in Virginia in the mid-1980’s (Reed, 2009). Initial adoption of this system was low; however, widespread acceptance of greenhouse tobacco transplant production was experienced throughout the 1990’s (Reed, 2009). There are several factors that influenced the rise in popularity of the greenhouse transplant production system, including reduced labor costs, greater control of environmental conditions, and more uniform plant growth (Reed, 2009).

In a float bed system, expanded polystyrene trays containing open-ended, inverted pyramidal cells filled with soilless media float on shallow water (Jones et al., 1993) in beds that are lined with black plastic. Nutrients are either placed in the float water and immediately mixed to distribute the nutrients or directly injected into the float water through the use of an injector system. To ensure proper growth, tobacco seedlings need to be exposed to sufficient amounts of macro-nutrients, such as nitrogen (N), phosphorus, and sulfur, and micro-nutrients, such as boron.
Among the elements essential for the commercial production of tobacco, none has as pronounced an effect nor requires the degree of attention in fertility practices as N (McCants and Woltz, 1967). According to Jones et al. (1993), it is suggested that at least 60% of the N be from a complete fertilizer, such as a 20-10-20 fertilizer, to ensure sufficient amounts of phosphorus and potassium. It would be possible to include N from sodium nitrate (16-0-0) to adjust for remaining N needed to produce a healthy plant. Nitrogen can exist in many different forms which can impact the availability for plant uptake. Organic N must be converted to ammonium ($\text{NH}_4^+$), through mineralization in order to be plant available. Other forms of N include urea and nitrate. Urea, however, cannot be assimilated by plants and therefore, it must be converted to ammonium before being available for plant uptake. Urease or ureolytic organisms present in float bed water can reduce toxicity by steadily decomposing urea (Pearce et al., 1998).

According to Vann and Fisher (2017), it is recommended that N levels in the float water solution consistently measure 125 parts per million (ppm). Higher application rates can produce tender, succulent seedlings that are more susceptible to diseases and more prone to fertilizer salts injury (Vann and Fisher, 2017).

Research at Clemson University has shown the need to limit phosphorus concentrations to 35-50 ppm in the water solution (Vann and Fisher, 2017). An excess of phosphorus will produce spindly transplants, while insufficient amounts can stunt growth (Vann and Fisher, 2017). Phosphorus levels are especially a concern with organic fertilizer sources derived from animal waste due to their naturally high amounts of phosphorus.

A proper fertility program is the key to producing healthy and uniform transplants. While N is the most critical nutrient, others such as phosphorus, play a large role even if they are not required in large concentrations. For organic flue-cured tobacco growers, managing fertility can
be very challenging due to the many different factors that impact nutrient release and availability with these organic sources. In general, organic fertilizers do not have large quantities of N in a plant available form and are typically high in phosphorus concentration and low in potassium.

**Organic N Fertilizer Sources**

Conventionally grown tobacco is fertilized with synthetic, inorganic fertilizers that are available for plant uptake, or become quickly available through hydrolysis reactions in the soil (Havlin et al. 2005). Nitrogen assimilated by plants may be in several forms, but nitrate (NO$_3^-$), ammonium (NH$_4^+$), and atmospheric N account for most N taken up in natural ecosystems and in agriculture (Franco and Munns, 1982). Nitrate and ammonium-N combined compile just 5% of the total amount of soil N, while the remaining 95% of the total soil N is organic and includes proteins, amino sugars, and nucleic acids. Generally speaking, organic fertilizers are low in N and potassium, yet high in phosphorus. Nitrogen, the most critical nutrient in tobacco transplant production, is often considered to be a limiting factor in the production healthy and uniform seedlings.

Providing sufficient soil N availability to reach maximum yield potential can be a challenge in organic culture (Hartz et al., 2010). This is a great concern amongst organic flue-cured tobacco producers as N impacts the yield and quality of a flue-cured tobacco crop more so than any other nutrient. To accommodate the high N demands that tobacco plants require, leguminous cover crops can be utilized to provide additional N to the soil. Cover cropping is generally the most economical way to provide plant-available N in organic systems (Gaskell and Smith, 2007). Other ways soil N is increased is from the decomposition of plant residues and soil organic matter, both of which are regarded as organic amendments that decompose at a relatively
slow rate (Hadas and Kautsky, 1994). However, for profitable crop production, farmers cannot rely on organic amendments as the sole source of available N, and need materials that release N at a greater rate for pre-planting or top fertilization (Hadas and Kautsky, 1994). There are different options available to growers that enable them to produce an organic tobacco crop with the sufficient amount of N. An application of manure-based compost is a common organic production practice, but the low N mineralization rate typical of compost limits its N contribution (Hartz et al. 2010). Hartz et al. (2000) reported that most compost mineralized less than 10% of initial N content in the four to six months following soil incorporation. A high compost application rate can make a significant contribution to soil N availability, but in practice, application rate is constrained by cost and by the potential for water quality degradation from high phosphorus loading (Sharpley et al., 1994). An alternative option available to organic flue-cured tobacco producers is the use of dry organic fertilizers such as fishery waste, feather meal, and seabird guano, all of which have high N content (>10% of dry weight) and relatively rapid N mineralization (Hartz et al. 2010). In contrast to manure-based compost, dry organic fertilizers mineralize 60% to 80% of N within four to eight weeks after application to agricultural soils (Hadas and Kautsky, 1994; Hadas and Rosenberg, 1992; Hartz and Johnstone, 2006). As previously mentioned, a popular dry organic fertilizer is seabird guano. Guano, an organic fertilizer, is a natural deposit of the excrement and remains of birds living along ocean coasts and feeding on fish (Hadas and Rosenberg, 1991). Guano has been an important fertilizer before industrial fixing of N from the air was developed (Hadas and Rosenberg, 1991). Nitrogen in organic fertilizers is claimed to be water insoluble, and it may be slowly released with the decay of the fertilizer (Cooke, 1972). However, guano was found to be nitrified in soil at a considerably more rapid rate than other organic fertilizers (Owen et al., 1950). While guano is N rich, rapidly
decomposable, and the most commonly used organic fertilizer, it is also an expensive material (Hadas and Kautsky, 1994). A considerably cheaper substitute for guano is feather meal. Feather meal, like guano, is also high in N content and is a by-product of poultry processing plants (Hadas and Kautsky, 1994). Ninety percent of feather dry weight consists of crude keratin protein, which, if processed by steam hydrolysis can increase immediate release of N, with most occurring in the first five weeks of incubation while microbial hydrolysis increased N release during the 8-11 week period (Jong-Myung and Nelson, 1995). The conversion of organic N to an inorganic form is essential for enabling plant uptake. That said, the N cycle can be very complex and the forms of N are very dynamic.

**Fates of N**

The potential sources of N in organic production are primarily composed of organic materials and occasionally small amounts of soluble nitrate or ammonium. Organic sources of N will go through a mineralization process in which soil microbes metabolize organic carbon and convert organic N into ammonium and a subsequent process that quickly oxidizes the ammonium to nitrate (Gaskell and Smith, 2007). Mineralization occurs through the activity of heterotrophic microorganisms that require organic carbon (C) for energy (Havlin et al., 2014). The mineralization of N to NH$_4^+$ occurs through two reactions, aminization and ammonification. Aminization converts proteins in residues to amino acids, amines and urea, which are all organic compounds that are further converted to inorganic NH$_4^+$ by ammonification (Havlin et al., 2014). The NH$_4^+$ that is produced through ammonification is subject to several fates, including nitrification, plant uptake, NH$_4^+$ fixation, volatilization, or immobilization (Havlin et al., 2014). Several factors that influence mineralization rates include, but are not limited to, soil pH, soil moisture, and soil temperature. Specifically, all three of the previously mentioned factors
influence aerobic and anaerobic microbial bacteria. In general, as pH increases, mineralization rates will also increase due to an increase in microbial activity. An argument can be made that soil moisture and temperature influence mineralization more so than other factors. Maximum aerobic activity and N mineralization occur between 50 and 70% water-filled pore space, while optimum soil temperature for microbial activity ranges between 25 and 35°C (Havlin et al., 2014). N mineralization is always coupled with immobilization in the soil environment, and a complex set of factors determines whether N is released for plant uptake or remains immobilized by soil microbes (Jarvis et al., 1996).

Nitrogen immobilization is the conversion of inorganic N to organic N (Havlin et al., 2014). If decomposing residues contain low N, microorganisms will immobilize NH$_4^+$ and NO$_3^-$ in the soil solution (Havlin et al., 2014). One of the main factors that influence immobilization is the ratio of carbon to N (C:N ratio). Microbes need N in a C:N ratio of roughly 8:1; therefore, inorganic soil N is utilized by the rapidly growing population, reducing NH$_4^+$ and NO$_3^-$ to very low levels (Havlin et al., 2014). While this can potentially cause plants to become N deficient, most cropping systems are exposed to sufficient N to compensate for N immobilization by microbes and crop N uptake (Havlin et al., 2014). Carbon: nitrogen ratios of materials used in organic production prove to be valuable information because ratios in soil organic matter are relatively constant, but if a material is added having a high C:N ratio (above 20-30) net N immobilization will occur due to increased microbial activity (Prasad and Power, 1997). If N immobilization does not occur, then the nitrification process will generally occur.

In a greenhouse float system, where soilless media is used, populations of beneficial organisms, such as nitrifying bacteria, are low or nonexistent (Pearce et al., 1998). Furthermore, nitrifying bacteria are known to be relatively scarce in peat, a major component in soilless media
(Herlihy, 1972). Despite low initial nitrifier populations in most soilless mixes, several studies show that the nitrification rate increases as a crop is grown in the media (Elliot, 1986; Lang and Elliot, 1991). According to Elliot (1986) and Lang and Elliot (1991), nitrification peaked at 4 to 6 weeks of cropping and then declined. Such patterns could influence the availability of N to plants and the accumulation of toxic components (Pearce et al., 1998). High fertilizer rates or a high proportion of NH$_4^+$ N may also decrease nitrification activity in the soilless media (Lang and Elliot, 1991). The presence of N in the ammonium form is the first requirement for nitrification to occur. In addition to ammonium, pH, aeration, moisture, temperature, and oxygen concentrations all impact nitrification (Ward, 2013). Although the oxidation of ammonium to nitrate takes place over a wide range in pH (5-9), optimum pH is roughly 8 (Havlin et al., 2014). Nitrifying bacteria need an adequate supply of calcium and micronutrients (Havlin et al., 2014). In addition to proper soil pH, adequate soil moisture is also required for optimal nitrification rates. Aerobic nitrifying bacteria will not produce nitrate in the absence of oxygen (Havlin et al., 2014). Soils that are coarse textured or possess good structure facilitate rapid gas exchange and ensure adequate supply oxygen for nitrifying bacteria (Havlin et al., 2014). Soil moisture and soil aeration of closely related when comparing their impacts on nitrification. Nitrification declines as soil moisture exceeds field capacity or nears air dryness (Havlin et al., 2014). In general, the more moisture that is present in a soil system, the less oxygen there is available for nitrifying bacteria. Soil temperature also plays a major role in the nitrification process. Although nitrification occurs over a wide temperature range, optimum soil temperature is 25-35°C (Havlin et al., 2014). Before nitrate is produced, through nitrification, organic fertilizers must first be converted to ammonium through the mineralization process. Nitrification is essential to produce
plant available N, but in anaerobic environments, such as a greenhouse float bed system, denitrification can occur.

The primary pathways of gaseous N losses are by denitrification and ammonia volatilization (Havlín et al., 2014). Large populations of denitrifying microorganisms exist, but the most common are the bacteria Pseudomonas, Bacillus, and Paracoccus, and several autotrophs (Havlín et al., 2014). Denitrification potential is high in most field soils, but certain conditions must be present to produce a shift from aerobic respiration to a denitrifying metabolism involving nitrate as an electron acceptor in the absence of conditions between years, between seasons, and within a given field (Havlín et al., 2014). The potential for denitrification and the magnitude of N loss is dependent on three things major things: whether the surface environment is waterlogged or anaerobic, whether nitrate and to a lesser extent nitrite is present in the anaerobic zone, and whether or not the surface soil contains an ample supply of decomposable or soluble carbon (Havlín et al., 2014). The occurrence of these three factors generally leads to a high denitrification potential and N loss.

Soil moisture content is critical to denitrification because of its effect on aeration (Havlín et al., 2014). In waterlogged environments, oxygen is excluded and anaerobic conditions occur. Generally, when soil water-filled pore space exceeds 60%, aerobic activity declines, while anaerobic microbial activity increases; however, appreciable N loss generally occurs at >80% water-filled pore space (Havlín et al., 2014). Denitrification accelerates under low oxygen diffusion in soil and a high microbial respiratory demand (high carbon source) (Havlín et al., 2014). When oxygen content of well-aerated soils, roughly 16-18%, declines to 8-10% oxygen, denitrification is optimized (Havlín et al., 2014). Nitrate must be present for denitrification to occur, and high nitrate increases denitrification potential (Havlín et al., 2014). However, nitrate
will be readily denitrified if anaerobic conditions occur after the initial fertilizer N application (Havlin et al., 2014). Nitrate is produced through the oxidation of ammonium to nitrate. This is a two-step process where ammonium is first converted to nitrite and then to nitrate. The source of ammonium can be from N mineralization, N fertilizers, or manures containing or forming ammonium. Nitrite generally does not accumulate in soils, which is fortunate since nitrite is toxic to plant roots (Havlin et al., 2014). In well aerated soils, nitrification occurs rapidly (Havlin et al., 2014). Similar to denitrification, nitrification is affected by many different factors including, supply of ammonium, soil pH, soil aeration, soil moisture, and soil temperature. Having N in the ammonium form is the first requirement for nitrification to occur. Although the conversion of ammonium to nitrate takes place over a wide range in pH (5-9), optimum pH is roughly 8 (Havlin et al., 2014). Nitrifying bacteria need an adequate supply of calcium and micronutrients (Havlin et al., 2014). In addition to proper soil pH, adequate soil moisture is also required for optimal nitrification rates. Aerobic nitrifying bacteria will not produce nitrate in the absence of oxygen (Havlin et al., 2014). Soils that are coarse textured or possess good structure facilitate rapid gas exchange and ensure adequate supply oxygen for nitrifying bacteria (Havlin et al., 2014). Soil moisture and soil aeration of closely related when comparing their impacts on nitrification. Nitrification is reduced when soil moisture exceeds field capacity or nears air dryness (Havlin et al., 2014). In general, the more moisture that is present in a soil system, the less oxygen there is available for nitrifying bacteria. Soil temperature also plays a major role in the nitrification process. Although nitrification occurs over a wide temperature range, optimum soil temperature is 25-35°C (Havlin et al., 2014).

Organic N must undergo several processes before becoming available for plant uptake. While N is often considered to be a limiting factor in organic seedling production, sufficient
amounts of several other macro and micro nutrients are also required. Research is needed to develop fertility programs which include all of the essential nutrients needed to produce healthy tobacco seedlings. By using a combination of N sources, such as N released from seabird guano and sodium nitrate, along with supplemental potassium, it is possible to produce tobacco seedlings that are comparable to seedlings produced with complete synthetic fertilizers. Addressing the challenges organic seedling producers face can provide answers to many questions that organic producers have regarding fertility management in a greenhouse float system. The research contained herein was designed to address the outlined challenges.

MATERIALS AND METHODS

Experimental Design and Greenhouse Procedures

From 2016-2017, a study was conducted to evaluate organic flue-cured tobacco seedling production in a float-bed system. The experiment was conducted at the Central Crops Research Station in Clayton, North Carolina. The greenhouse used in this study is typical of what is used by tobacco producers in North Carolina. The objective of this study was to compare a conventional complete fertilizer source (SQM, 16-5-15) to multiple organic fertilizer sources consisting of sodium nitrate (SQM-Allganic, 16-0-0), pelletized Peruvian seabird guano (Sunleaves, 12-11-2), or a combination of both, and to quantify nutrient availability and seedling growth. In total, 7 treatments were developed with each treatment being replicated 3 times.

To provide adequate growing space and to separate treatments, 21 mini beds were constructed inside of the greenhouse with each mini bed designed to hold six 288 cell expanded polystyrene (EPS) trays. Each tray had the center 100 cells of the total 288 cells marked off for
data collection throughout the study. Trays were filled with organic soilless media provided by Carolina Greenhouse located in Kinston, North Carolina and seeded on June 4, 2016 and November 9, 2016 with variety K 326 and then floated in the designated bed. One seed was placed per cell. All beds were filled with water from a deep well to a depth of 13.7 cm. All fertilizers were applied directly into the solution of each individual bed 9 and 21 Days After Seeding (DAS). When guano was included in a treatment, it was ground to pass through a 1 mm sieve in a food processor to increase surface area and solubility. Prior to applying the fertilizer, all components of each fertility program were placed in a sterile, 474 mL hard-plastic bottle designated for each individual treatment. Hot tap water (50 °C) was then added to each bottle and then each bottle was shaken for two minutes to dissolve the fertilizer. The dissolved components were transferred to the greenhouse and then evenly distributed in the appropriate mini bed. This process was repeated for the second fertilizer application.

Nitrogen, phosphorus (P) and potassium (K) nutrient rates targeted 125, 40, and 125 mg/L, respectively, for the complete 16-5-16 conventional fertility program, the seabird guano plus sodium nitrate program, and seabird guano and sodium nitrate plus gypsum. When seabird guano was applied as the exclusive source of N, 125 mg/L of N and K were targeted. Targeting this rate of N for these two treatments resulted in 115 mg P/L. Treatments which used sodium nitrate as the exclusive source of N targeted 125 mg/L of N and K; however, P was not included in these two treatments. Where gypsum is included in the fertility program, 50 mg Ca/L was targeted.

Throughout the study, nutrient availability was quantified to measure the differences among treatments. Every five days the water depth was elevated to 13.7 cm in each bed and then a solution sample was collected from all 21 mini beds. Solution samples were collected in a
sterile hard-plastic bottle and were immediately transferred to North Carolina Department of Agriculture and Consumer Services-Agronomic Division for analysis. In addition to collecting solution samples, dissolved oxygen was also measured every five days using a YSI Professional Plus dissolved oxygen meter. Further data collected included percent spiral root and soluble salt injury, which was collected 15 and 20 DAS, while percent germination was solely collected at 15 DAS. A percentage for each of the previous data categories was obtained by examining the middle 100 cells from each individual tray. Each percentage from each individual tray was then added and then divided by six, the number of trays in each mini bed. Furthermore, a media sample was collected 20 DAS from within the center 100 cells that were marked off and used for data collection. Fifteen media samples were collected from each tray for a total of 75 samples per treatment. A second media sample was collected at the conclusion of each study and following the same procedures. Media samples, along with tissue samples, were collected and immediately taken to North Carolina Department of Agriculture and Consumer Services Agronomic Division for analysis. In addition to collecting media samples at the conclusion of the study, ten whole plant tissue samples were also collected from each bed, detached from the soilless media, and then measured for stem height and diameter. Stem height was measured from the base of the stem to the bud, while stem diameter was measured using a caliper. Stem diameter measurements were collected from the first node above the media line. In addition, at the conclusion of the study the total plant stand was quantified and then the amount of usable transplants was recorded. Total plants only included plants that were within the 100 cell barrier that was marked at the beginning of the study. Usable transplants were defined as transplants that would be healthy enough to be mechanically transplanted and survive field conditions. Each tray was clipped a minimum of five days throughout the transplant production season. Previous research
has shown that maximum usability is obtained with three to five clippings (Fisher and Vann, 2017). Improper clipping can reduce stem length, increase stem rots, and slows plant growth in the field (Fisher and Vann, 2017).

**Analytical Procedures**

**Solution Samples**

*Nitrogen.* The inorganic N fraction concentrations include nitrate plus nitrite and ammonium. The organic N fraction concentration includes urea. Nitrate is determined on ~10 mL homogenized sample, filtered using acid washed filter paper (Laboratory Filtration Group, Houston, TX) by nitrate-hydrazine reduction (Kempers 1988; Skalar Analytical 1995a); NH$_4$-N is determined by a modified Berthelot reaction (adapted from Krom 1980; Skalar Analytical 1995b); and urea concentration is determined with the diacetyl monoxime thiosemicarbazide colorimetric method (Sullivan and Havlin, 1991; Skalar Analytical 1995, Issue 6) with an auto-flow spectrophotometric analyzer (San++ Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands).

*pH.* The pH values are measured directly on homogenized samples. The pH is measured using a hydrogen electrode (Orion 920A; Thermo Fisher Scientific; Beverly, MA) (Eaton et al., 2005).

*Bicarbonate.* Bicarbonate concentrations are measured by titration using phenolphthalein and methyl orange as endpoint indicators (AOAC, 1990). Results of bicarbonate are expressed in units of meq HCO$_3^-$ L$^{-1}$.
Media Samples

Samples were analyzed as received by the North Carolina Department of Agriculture and Consumer Services – Agronomic Division. pH is measured on a 1:1 v/v ratio of fresh sample to DI water following a 60 minute equilibration. The saturated media extract is obtained by adding DI water to a fresh ~400 mL sample until it reaches a saturation point. The sample is allowed to equilibrate for 60 minutes, and then the liquid is extracted from the sample by vacuum filtration (Whatman 1 Qualitative Circles, 110 mm).

*Nitrogen.* Inorganic-N fraction concentrations include nitrate-N plus nitrite-N and ammonium-N. Organic N fraction concentration includes urea. Nitrate N is determined on the saturated media extract, (~10 mL) by nitrate-hydrazine reduction (Kempers 1988; Skalar Analytical 1995a); Ammonium N is determined by a modified Berthelot reaction (adapted from Krom 1980; Skalar Analytical 1995b); and urea concentration is determined with the diacetyl monoxime thiosemicarbazide colorimetric method (Sullivan and Havlin, 1991; Skalar Analytical 1995, Issue 6) with an auto-flow spectrophotometric analyzer (San++ Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands).

*pH.* The pH values are measured on a 1:1 v/v ratio of fresh sample to DI water following a 60 minute equilibration. The pH is measured using a hydrogen electrode (Orion 920A; Thermo Fisher Scientific; Beverly, MA) (Eaton et. al., 2005).

RESULTS AND DISCUSSION

This study was conducted twice, each time in a separate environment. Each environment was analyzed and is reported individually due to differences in duration. Data collected for total
plants, usable plants, stem height and stem diameter was collected after each tray had been mowed a minimum of five times, which marked the conclusion of each study.

**Solution Analysis**

*Bicarbonates.* In environments one and two (Figure 1), bicarbonate concentration is greatest in treatments that contain seabird guano. The highest bicarbonate concentrations were observed in treatments where seabird guano was the sole source of N (Figure 1). For fertility treatments containing at least a portion of seabird guano, it was observed that bicarbonate concentrations in both environment one and environment two peaked around 25 days after seeding (Figure 1). Results indicate that bicarbonate concentration decreases when less seabird guano is applied as part of the fertility program. In treatments where seabird guano was completely absent from the fertility program, bicarbonate concentration was never greater than 1 meq/L in both environments (Figure 1). This observation suggests that bicarbonates were being released or possibly created from the seabird guano. Total and usable plants produced in each environment did not appear to be affected by the presence of bicarbonates, even in environment two, where bicarbonate concentration surpassed 14 meq/L 25 days after seeding. Bicarbonate concentration can increase the pH of solution water and therefore reduce nutrient uptake by plants. Buffering capacity could explain why bicarbonate toxicity symptoms were not observed in this study. Seabird guano, which is high in NH$_4^+$ concentration, would release hydrogen (H$^+$) ions into the float water solution as it oxidizes to form NO$_3^-$. The additional H$^+$ ions released into solution would serve as an acid and buffer against the high concentrations of HCO$_3^-$. In this scenario it is plausible that the H$^+$ ions maintained the pH at a value where nutrient uptake was not diminished to a point where plant growth was reduced. Previous research conducted on *L.*
*microphyllum* has shown where plants grown in pH 8.0 produced 50% of the biomass compared to *L. microphyllum* grown in pH’s of 5.5 and 6.5 (Soti et al., 2015).

**Dissolved Oxygen.** In Figure 2, dissolved oxygen concentration from each environment was consistently lowest in treatments where seabird guano was the only source of N, as opposed to being coupled with sodium nitrate (16-0-0). This observation suggests the nutrients being released from the seabird guano are depleting the solution of oxygen. Microorganisms that decompose organic matter require dissolved oxygen (Utah State University, 2016). Therefore, high amounts of organic waste can lead to microorganisms using more oxygen than can be replaced in the environment (Utah State University, 2016). Reducing the amount of seabird guano plus including sodium nitrate can lead to an increased dissolved oxygen concentration. However, treatments that contained both seabird guano and sodium nitrate still maintained a lower presence of dissolved oxygen in both environments compared to treatments where seabird guano was not incorporated into the fertility program (Figure 1). Environment one shows a steady decrease in the overall dissolved oxygen concentrations for each treatment throughout the study, while environment two shows concentrations remaining relatively constant for certain treatments (Figure 1). This could be attributed to a longer, cooler growing environment compared to environment one.

**Nitrate N.** In environments one and two (Figure 3) nitrate concentrations in the float bed solution were highest when fertility treatments included sodium nitrate as the exclusive source of N. Initially, there is <5 mg NO₃⁻ L⁻¹ of nitrate present in both environments where seabird guano is the exclusive N source. However, between 25 and 35 DAS, a rapid increase in nitrate is observed in these same treatments (Figure 3). It is plausible that exposing the seabird guano to
these anaerobic conditions for a prolonged period of time allows the nitrification rate to increase, thus leading the ammonium-N oxidation into nitrate-N.

Ammonium N. In environment one and two (Figure 4), ammonium concentration is highest throughout the entirety of the study for treatments containing seabird guano as the exclusive source of N. This is likely due to the organic form of N present in the seabird guano mineralizing to form high amounts ammonium prior to nitrification. Data from Environment 1 and 2 show an increase in nitrate and a decrease in ammonium beginning 30 DAS (Figures 3 & 4). It is possible this trend was observed due to nitrification rates increasing after the organic N in the seabird guano was mineralized to form ammonium. Initially, the N from seabird guano is in the organic form, which is composed of stable and unstable N forms, such as urine and feces, respectively (Havlin et al., 2014). While urine, which includes, amino acids, urea and some ammonium, can undergo a rapid mineralization, feces, which includes proteins and carbohydrates, mineralizes at a slower rate (Havlin et al., 2014). This could explain the initial increase in ammonium and eventual increase in nitrate. Given time, it is possible the proteins and carbohydrates will further mineralize and ultimately produce nitrate.

Inorganic N. Inorganic N is the combination of ammonium, nitrate, and nitrite N. In environment one and two (Figure 5) total inorganic N peaks after each fertilizer application. Following the second fertilizer application 21 days after seeding, inorganic N steadily decreases in both environments (Figure 5). One explanation for this observation is N being assimilated by plants, thus depleting the inorganic N from the solution. Dilution from the addition of tap water every 5 days could also result in less inorganic N measured in the solution. Treatments containing sodium nitrate as the sole source of N had the highest amount of inorganic N at the final sampling interval in both environments (Figure 5). It is possible that the organic N in the
seabird guano did not have time to fully mineralize throughout the study. Results show treatments containing at least a portion of seabird guano have more urea remaining in solution (Figure 7). This would indicate that not the entire organic N from the seabird guano has been allowed to mineralize, which would decrease the amount of inorganic N present in solution.

**pH.** With the exception of the conventional 16-5-16 fertilizer, the pH values were relatively consistent in both environments (Figure 6) throughout the duration of the study. The pH of the solution water can potentially control many different aspects of nutrient release and availability. Bicarbonate concentration also impacts solution pH, and pH was observed to be highest in the two treatments where seabird guano was the exclusive source of N (Figure 6). These two treatments also produced the greatest bicarbonate concentration (Figure 1). It is possible that the bicarbonates in solution are buffering against the pH and therefore, keeping the pH higher in treatments containing seabird guano.

**Urea.** Urea concentration in both environments was highest in treatments that contained seabird guano as a portion of the fertility program (Figure 7). These treatments also had the greatest concentration of bicarbonate in the float water solution (Figure 1). It is possible that there is a correlation between urea concentration and bicarbonate concentration. Urease is a nickel-dependent enzyme, present in various plants and microorganisms that catalyzes the hydrolysis of urea to ammonia and carbon dioxide, which causes an abrupt increase of pH (Karplus et al., 1997; Zerner, 1991). Once CO$_2$ is released, it can bind with free H$^+$ ions to form HCO$_3^-$, which would result in an increase in pH. Lower urea and bicarbonate concentrations were observed in treatments not containing seabird guano as a portion of the fertility program (Figures 1 & 7).
Seedling Measurements

Data collected from seedlings occurred after each tray had been clipped a minimum of five times. Clipping a minimum of five times allows smaller plants to catch up with the larger plants with respect to size and increases stem diameter (Fisher and Vann, 2017). Too many clippings indicates that the greenhouse was seeded too early (Fisher and Vann, 2017). Current recommendations are to begin clipping at three to five day intervals when total plant height is two to 2.3 inches about the tray and to set the blade at one to 1.5 inches above the bud (Fisher and Vann, 2017).

Total Plants. Five of the seven treatments successfully produced seedlings (Table 1). The two treatments that did not produce any seedlings did not have phosphorus included in the fertility program, which further emphasizes the importance phosphorus has on the growth and development of tobacco transplants. These two treatments, sodium nitrate plus 0-0-52 and sodium nitrate plus 0-0-52 and gypsum, were not included in the data analysis. The remaining five treatments produced a high percentage of total plants. No statistical differences were observed with respect to total plants produced (Table 1).

Usable Plants. Usable plants were evaluated by determining whether or not the plant would be likely to be successfully transplanted with a mechanical transplanter, survive field conditions and survive until maturity. Five of the seven treatments produced a high percentage of usable plants (Table 1). These five fertilizers targeted 125 ppm N and K plus 40 ppm P. A complete fertility program is critical in producing usable transplants. The two treatments that incorporated sodium nitrate and seabird guano as the sources of N produced the highest number of usable plants. Incorporating these two sources of N provided a more balanced ammonium: nitrate concentration in the float water solution.
**Stem Height.** Stem height was measured from the base of the stem to the base of the bud. Little differences were observed in stem heights (Table 1). Differences observed in stem height would likely have little to no agronomic impact. The seabird guano plus 0-0-52 treatment yielded a slightly shorter plant, but did not impact total plants or usable plants that were produced by this treatment.

**Stem Diameter.** Research from 2016 and 2017 show little differences in stem diameter (Table 1); however, two treatments, seabird guano plus 0-0-52 and seabird guano plus 0-0-52 and gypsum, exhibited smaller stem diameters. Differences observed in stem diameter would likely have little to no agronomic impact and, in addition, stem diameter did not seem to impact total plants or usable plants produced.

**Soilless Media Analysis**

Soilless media samples were collected at two different intervals. Data for each sampling interval were pooled across both environments.

**20 Days After Seeding.** Six different nutrients within the soilless media were analyzed 20 days after seeding (Tables 2, 3, & 4). Results from research conducted in 2016-2017 show treatments consisting of seabird guano as the sole source of nitrogen had a high concentration of ammonium (Tables 3 & 4). It is preferable to have a balanced ratio of ammonium: nitrate; however, given time to nitrify, ammonium in the media should oxidize to form nitrate. A proper ammonium: nitrate balance has been found to be 1:3 (Thorpe et al. 1989).

pH in the media was also highest in treatments only containing seabird guano (Tables 3 & 4). This correlates with the solution pH 20 days after seeding, which show solution pH is highest in treatments containing seabird guano without sodium nitrate (Figure 6). While
bicarbonate concentration was not measured in the soilless media, it is possible that the pH in treatments containing seabird guano is greatest due to bicarbonate presence within the soilless media.

Nitrate was observed in high concentrations in all treatments containing sodium nitrate as a portion of the fertility program (Tables 3 & 4). These treatments were also highest in soluble salts (Table 2). This could be explained by evapotranspiration, which would result in concentrations of nutrients in the soilless media. Seedlings may not have assimilated much of the nitrate in the medium because seedlings typically will not uptake more nutrients than needed. However, it is also possible that high concentrations of soluble salts observed in sodium nitrate based treatments negatively impacted nitrate assimilation and therefore resulted in a high concentration of nitrate in the growing medium.

End of Study. Similar trends were observed in the soilless media samples collected at the conclusion of the study as were seen 20 days after seeding (Tables 2, 3, & 4). Treatments devoid of seabird guano contained the highest amounts of inorganic N in the media, while also containing the highest concentration of soluble salts (Tables 2 & 3). The two treatments consisting of only seabird guano as the source of nitrogen show higher amounts of nitrate and lower amounts of ammonium compared to samples collected 20 days after seeding. This is likely attributed to the ammonium undergoing nitrification.

It was observed that nitrate concentration increased in the soilless media from the 20 DAS sampling interval (Table 3) in treatments where sodium nitrate was applied as the exclusive form of N. These two treatments did not contain any phosphorus and at the conclusion of the study and seedlings became necrotic. This would have negatively impacted nitrate assimilation and resulted in an increased concentration of nitrate and inorganic nitrogen in the soilless media.
CONCLUSION

Maintaining the recommended concentration of specific nutrients, specifically N, is critical for the production of healthy, usable tobacco seedlings. Furthermore, a balanced supply of ammonium and nitrate N is preferable. While there are concerns, such as high bicarbonate concentrations, with some of the treatments used in this study, none of these factors negatively impacted plant growth.

The results from 2016 and 2017 demonstrate that the two treatments containing seabird guano as the exclusive source of N produced a more imbalanced ammonium to nitrate ratio. In addition, a high bicarbonate concentration was observed in the float bed solution where these two treatments were utilized. It is possible that a greater concentration of urea being produced from the seabird guano undergoes a chemical reaction that produces $\text{HCO}_3^-$ ions. Adding sodium nitrate to minimize the amount of seabird bird guano needed to achieve 125 ppm N proved to be a suitable alternative. Results demonstrate that this practice can increase nitrate concentration, and decrease ammonium and bicarbonate concentration in the float water solution. The best treatment, based on the ammonium to nitrate ratio and usable plants produced, was observed when sodium nitrate was applied with seabird guano, sulfate of potash (0-0-52), and gypsum.

Ultimately, five of seven treatments produced healthy and usable tobacco transplants. Having a fertility program that targets appropriate amounts of macro and micro nutrients is an important aspect of tobacco transplant production. Understanding the differences and challenges of organic transplant production, particularly nutrient availability and release, is an important step to producing high quality transplants. Utilizing the information from this study can help growers further understand some of the challenges they may face, and may also provide suggestions to organic transplant producers.
Literature Cited


Table 1. Total transplants, usable transplants, transplant height, and transplant diameter reported by corresponding treatment as affected by fertility programs\textsuperscript{a,b,c}.

<table>
<thead>
<tr>
<th>Treatment List\textsuperscript{c}</th>
<th>Total Plants</th>
<th>Usable Plants</th>
<th>Stem Diameter</th>
<th>Stem Height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em><strong>-</strong></em>%___</td>
<td>___-<em><strong>mm</strong></em></td>
<td>___-<em><strong>cm</strong></em></td>
<td></td>
</tr>
<tr>
<td>16-5-16</td>
<td>88 a</td>
<td>79 bc</td>
<td>3.30 a</td>
<td>6.62 a</td>
</tr>
<tr>
<td>SG\textsuperscript{c} + 0-0-52</td>
<td>90 a</td>
<td>78 c</td>
<td>2.76 b</td>
<td>5.62 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>91 a</td>
<td>85 ab</td>
<td>3.48 a</td>
<td>6.27 ab</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>93 a</td>
<td>86 a</td>
<td>3.43 a</td>
<td>6.47 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>91 a</td>
<td>79 bc</td>
<td>2.84 b</td>
<td>6.08 ab</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter within the same column are not significant at the $\alpha=0.05$ level. Data are pooled across two environments.

\textsuperscript{b}Treatments comprised of 16-0-0 and 0-0-52 removed from analysis.

\textsuperscript{c}16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Seabird Guano (Sunleaves 12-11-2)
Table 2. Soilless media nutritional concentration 20 days after seeding*.

<table>
<thead>
<tr>
<th>Treatments**</th>
<th>Urea</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>mS/cm</td>
</tr>
<tr>
<td>16-5-16</td>
<td>0.19 ab</td>
<td>174.17 e</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>0.17 b</td>
<td>172.17 e</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>0.28 ab</td>
<td>315.17 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>0.31 ab</td>
<td>240.50 d</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>0.11 b</td>
<td>359.17 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>0.15 b</td>
<td>279.17 c</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>0.47 a</td>
<td>189.50 e</td>
</tr>
</tbody>
</table>

*Treatment means followed by the same letter within the same column are not statistically different at the α=0.05 level.

**16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
Table 3. Soilless media nutritional concentration from Environment 1 collected 20 days after seeding\(^a\).

<table>
<thead>
<tr>
<th>Treatments(^b)</th>
<th>Inorganic Nitrogen</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16</td>
<td>128.67 cd</td>
<td>22.97 c</td>
<td>105.80 c</td>
<td>5.16 c</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>50.03 d</td>
<td>51.6 b</td>
<td>0.41 d</td>
<td>5.59 b</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>281 a</td>
<td>1.04 d</td>
<td>280 a</td>
<td>5.17 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>130.33 bc</td>
<td>20.37 c</td>
<td>110 bc</td>
<td>5.53 b</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>278.33 a</td>
<td>1.19 d</td>
<td>277 a</td>
<td>5.22 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>163.33 b</td>
<td>23.30 c</td>
<td>140 b</td>
<td>5.48 b</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>65.40 d</td>
<td>63.50 a</td>
<td>1.89 d</td>
<td>6.00 a</td>
</tr>
</tbody>
</table>

\(^a\)Treatment means followed by the same letter within the same column are not statistically different at the \(\alpha=0.05\) level. Data are reported by individual environment.

\(^b\) 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
Table 4. Soilless media nutritional concentration from Environment 2 collected 20 days after seeding\(^a\).

<table>
<thead>
<tr>
<th>Treatments(^b)</th>
<th>Inorganic Nitrogen</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16</td>
<td>17.67 b</td>
<td>17.27 b</td>
<td>0.42 c</td>
<td>5.45 bc</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>43.60 b</td>
<td>42.97 a</td>
<td>0.61 c</td>
<td>5.86 a</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>186.33 a</td>
<td>0.83 c</td>
<td>185.33 ab</td>
<td>5.50 bc</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>158.33 a</td>
<td>22.37 b</td>
<td>136.33 b</td>
<td>5.66 ab</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>216.33 a</td>
<td>1.02 c</td>
<td>215.33 a</td>
<td>5.34 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>151.67 a</td>
<td>20.67 b</td>
<td>130.80 b</td>
<td>5.62 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>39.37 b</td>
<td>38.87 a</td>
<td>0.57 c</td>
<td>5.79 a</td>
</tr>
</tbody>
</table>

\(^a\)Treatment means followed by the same letter within the same column are not statistically different at the \(\alpha=0.05\) level. Data are reported by individual environment.

\(^b\) 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
### Table 5. Soilless media nutritional concentration at the conclusion of the study\(^a\).

<table>
<thead>
<tr>
<th>Treatments(^b)</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Urea</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16</td>
<td>33.28 a</td>
<td>14.98 bc</td>
<td>0.11 a</td>
<td>5.12 c</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>36.88 a</td>
<td>3.44 c</td>
<td>3.76 a</td>
<td>5.16 c</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>2.75 b</td>
<td>386.00 a</td>
<td>0.40 a</td>
<td>6.15 ab</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>6.25 b</td>
<td>46.73 b</td>
<td>0.26 a</td>
<td>6.60 a</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>3.47 b</td>
<td>383.33 a</td>
<td>0.12 a</td>
<td>5.90 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>9.80 b</td>
<td>34.30 bc</td>
<td>0.13 a</td>
<td>5.93 b</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>44.97 a</td>
<td>3.42 c</td>
<td>0.03 a</td>
<td>4.91 a</td>
</tr>
</tbody>
</table>

\(^a\)Treatment means followed by the same letter within the same column are not statistically different at the \(\alpha=0.05\) level. Data was combined across two environments.

\(^b\) 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
Table 6. Soilless media nutritional concentration from Environment 1 collected at the conclusion of the study.

<table>
<thead>
<tr>
<th>Treatments b</th>
<th>Inorganic Nitrogen</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16</td>
<td>81.43 b</td>
<td>177.33 c</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>47.07 b</td>
<td>250.00 bc</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>373.33 a</td>
<td>440.67 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>46.80 b</td>
<td>249.00 bc</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>346.67 a</td>
<td>465.33 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum</td>
<td>47.07 b</td>
<td>322.00 b</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>62.13 b</td>
<td>282.00 b</td>
</tr>
</tbody>
</table>

a Treatment means followed by the same letter within the same column are not statistically different at the α=0.05 level. Data are reported by individual environment.

b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
Table 7. Soilless media nutritional concentration from Environment 2 collected at the conclusion of the study.a.

<table>
<thead>
<tr>
<th>Treatmentsb</th>
<th>Inorganic Nitrogen</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16</td>
<td>--ppm--</td>
<td>--mS/cm--</td>
</tr>
<tr>
<td>SG + 0-0-52</td>
<td>26.97 b</td>
<td>175.33 e</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52</td>
<td>33.63 b</td>
<td>234.00 d</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52</td>
<td>403.67 a</td>
<td>500.33 b</td>
</tr>
<tr>
<td>16-0-0 + 0-0-52 + Gypsum</td>
<td>54.20 b</td>
<td>276.67 d</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 +Gypsum</td>
<td>427.33 a</td>
<td>595.67 a</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum</td>
<td>33.44 b</td>
<td>338.00 c</td>
</tr>
<tr>
<td></td>
<td>32.33 b</td>
<td>254.33 d</td>
</tr>
</tbody>
</table>

a Treatment means followed by the same letter within the same column are not statistically different at the α=0.05 level. Data are reported by individual environment.

b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

Table 8. P values for inorganic nitrogen, ammonium nitrogen, nitrate nitrogen, urea, soluble salts, and pH for media samples collected 20 days after seeding.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Environment (E)</th>
<th>Rep (E)</th>
<th>Treatment (Trt)</th>
<th>E x Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic N</td>
<td>0.0002</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>0.0048</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Ammonium</td>
<td>0.0012</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>0.0022</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.0003</td>
<td>0.0254</td>
<td>&lt;0.0001</td>
<td>0.0011</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>pH</td>
<td>0.0017</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0485</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>0.0116</td>
<td>---</td>
</tr>
<tr>
<td>Soluble Salts</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 9. P values for inorganic nitrogen, ammonium nitrogen, nitrate nitrogen, urea, soluble salts, and pH for media samples collected at the conclusion of the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Environment (E)</th>
<th>Rep (Environment)</th>
<th>Treatment (Trt)</th>
<th>E x Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic N</td>
<td>NS</td>
<td>0.0051</td>
<td>&lt;0.0001</td>
<td>0.0170</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>0.0672</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Soluble Salts</td>
<td>0.0232</td>
<td>0.0333</td>
<td>&lt;0.0001</td>
<td>0.0143</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Ammonium</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Nitrate</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>pH</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 10. Baseline solution and media nutrient concentrations collected from Environment 1 prior to seeding.

<table>
<thead>
<tr>
<th></th>
<th>Inorganic N</th>
<th>Urea</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Bicarbonate</th>
<th>Phosphorus</th>
<th>pH</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media</strong></td>
<td>9.00</td>
<td>0.35</td>
<td>3.26</td>
<td>5.74</td>
<td>--</td>
<td>0.13</td>
<td>4.56</td>
<td>106.80</td>
</tr>
<tr>
<td><strong>Solution</strong></td>
<td>3.96</td>
<td>0.54</td>
<td>0.55</td>
<td>3.41</td>
<td>0.30</td>
<td>0.09</td>
<td>6.12</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 11. Baseline solution and media nutrient concentrations collected from Environment 2 prior to seeding.

<table>
<thead>
<tr>
<th></th>
<th>Inorganic N</th>
<th>Urea</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Bicarbonate</th>
<th>Phosphorus</th>
<th>pH</th>
<th>Soluble Salts</th>
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<td><strong>Media</strong></td>
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<td>0.10</td>
<td>1.20</td>
<td>44.50</td>
<td>--</td>
<td>0.10</td>
<td>4.37</td>
<td>162.75</td>
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<tr>
<td><strong>Solution</strong></td>
<td>4.12</td>
<td>0.12</td>
<td>0.51</td>
<td>3.61</td>
<td>0.35</td>
<td>0.09</td>
<td>6.21</td>
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</tr>
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</table>
Figure 1. Bicarbonate concentration of the float water solution as affected by fertility program. Data are reported by individual environment. Dotted line represents bicarbonate threshold at 2 meq HCO$_3^-$, above which, seedling toxicity can occur.
Figure 2. Dissolved Oxygen (DO) concentration of the float water solution as affected by fertility program. Data are reported by individual environment.
Figure 3. Nitrate concentration of the float water solution as affected by fertility program. Data are reported by individual environment.
Figure 4. Ammonium concentration of the float water solution as affected by fertility program. Data are reported by individual environment.
Figure 5. Inorganic nitrogen concentration of the float water solution as affected by fertility program. Data are reported by individual environment.
Figure 6. pH of the float water solution as affected by fertility program. Data are reported by individual environment.
Figure 7. Urea concentration in the float water solution. Data are reported by individual environment.
Chapter Three

Impact of Aeration on Organic Fertility Programs for Flue-cured Tobacco (*Nicotiana tabacum* L.) Seedling Production.

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ABSTRACT

Organic flue-cured tobacco (*Nicotiana tabacum* L.) production has experienced significant expansion. Despite this expansion, there is very little information available that outlines organic fertility programs for seedling production. To provide this information, research was conducted at Central Crops Research Station (CCRS) in Clayton, North Carolina to evaluate the effect of organic fertility programs to seedling vigor and float water chemistry. In addition, the presence of aeration was evaluated to determine the impact additional oxygen presence may have on nutrient release and availability. This study was replicated twice between June 2016 and January 2017.

The objective of this study was to compare a conventional 16-5-16 fertilizer to organic nitrogen sources including Peruvian seabird guano, sodium nitrate (16-0-0), or a combination of both. A total of five treatments were created to observe differences between fertility programs and each treatment was replicated three times. Each treatment was placed into non-aerated and aerated mini beds. A Hydor bubble maker was placed in the middle of appropriate mini beds to provide additional oxygen to the environment. Additional oxygen could potentially increase mineralization and nitrification rates of the organic fertilizer sources. Data collected throughout the entirety of the growing season included solution samples, media samples, water temperature, dissolved oxygen, stem height, stem diameter, total plants, and total usable plants.

Research conducted from 2016-2017 demonstrates that aeration can have a significant role in nutrient release and availability. It was observed that aeration increased the amount of nitrate and decreased the amount of ammonium present in the float bed solution where seabird guano was applied as the exclusive source of N. In addition, it was observed that treatments containing at least a portion of seabird guano contained higher amounts of bicarbonates than
those absent of the N source. Bicarbonate concentration was reduced when these treatments were applied to an aerated solution. Overall, all treatments produced an acceptable percentage of usable plants. Data from this study can potentially be used to provide successful options for organic tobacco transplant producers in North Carolina.
NOMENCLATURE

*Nicotiana tabacum* L., tobacco

KEY WORDS

nitrogen, organic, fertility, aeration

INTRODUCTION

**Flue-cured Tobacco Transplant Fertility**

Commercial greenhouse production of tobacco transplants first appeared in Virginia in the mid-1980’s (Reed, 2009). Initial adoption of this system was slow, widespread acceptance of greenhouse tobacco transplant production was experienced throughout the 1990’s (Reed, 2009). Greenhouse-produced tobacco seedlings are often grown in cells in non-degradable trays resulting in intact roots after removal (Jones et al., 1993). There are several factors that influenced the rise in popularity of the greenhouse transplant production system, including reduced labor costs, greater control of environmental conditions, and more uniform plant growth.

In a float bed system, expanded polystyrene trays containing open-ended, inverted pyramidal cells filled with soilless media float on shallow water (Jones et al., 1993) in beds that are lined with black plastic. Nutrients are either placed in the float water and immediately mixed to distribute the nutrients or directly injected into the float water through the use of an injector system. To ensure proper growth, tobacco seedlings need to be exposed to sufficient amounts of
macro-nutrients, such as nitrogen (N), phosphorus, and sulfur, and micro-nutrients, such as boron.

Among the elements essential for the commercial production of tobacco, none has as pronounced an effect nor requires the degree of attention in fertility practices as nitrogen (N) (McCants and Woltz, 1967). According to Jones et al. (1993), it is suggested that at least 60% of the nitrogen be from a complete fertilizer, such as a 16-5-16 fertilizer, to ensure sufficient amounts of phosphorus and potassium. Nitrogen can exist in many different forms which can impact the availability for plant uptake. Organic N must be converted to ammonium (NH$_4^+$) through mineralization in order to be plant available. Other forms of nitrogen include urea and nitrate. Urea, unlike NH$_4^+$ and NO$_3^-$, cannot be assimilated by plants and therefore, it must be converted to NH$_4^+$ before being available for plant uptake. Urease or ureolytic organisms present in float bed water can reduce toxicity by steadily decomposing urea (Pearce et al., 1998).

Another organic nitrogen source that is used to produce organic flue-cured tobacco transplants is sodium nitrate (16-0-0). This provides nitrogen in the nitrate form, which is immediately available for plant uptake. However, sodium nitrate can account only for 20% of your total nitrogen applied to the float-bed solution throughout the transplant production period (Vann and Inman, 2017). Regardless of nitrogen source, there should always be an appropriate amount of nitrogen present to produce healthy transplants. According to Fisher and Vann (2017), it is recommended that N levels in the float water solution consistently measure 125 parts per million (ppm). Higher application rates can cause tender, succulent seedlings that are more susceptible to diseases and more prone to fertilizer salts injury (Fisher and Vann, 2017). Tobacco seedlings, in addition to N, need a sufficient amount of phosphorus for proper growth and development.
Research at Clemson University has shown the need to limit phosphorus concentrations to 35-50 ppm in the waterbed (Fisher and Vann, 2017). An excess of phosphorus will produce spindly transplants, while insufficient amounts can stunt growth (Fisher and Vann, 2017). Phosphorus levels are especially a concern with organic fertilizer sources due to their naturally high amounts of phosphorus compared to complete fertilizers used to produce conventionally grown tobacco transplants. Phosphorus has various roles within a plant. It plays a very important role in various metabolic processes, being a constitute of nucleic acids, phospholipids, coenzymes activating the amino acid production used in protein synthesis, DNA, RNA and ATP (Rouached et al., 2010). When there is a phosphorus deficiency, it is related with the reduction of chloroplast carbon fixation as a consequence to the photosynthetic potential (Chrysargynis et al., 2016). A study by Chrysargynis (et al., 2016) was conducted to determine the effects of nitrogen and phosphorus levels on the biological and morphological characteristics of lavender under hydroponic conditions. This study revealed high phosphorus concentrations positively affected the plant growth parameters. Plants grown under the highest phosphorus concentration (70 mg/L P) revealed the greatest biomass (19.47 g/plant), while plants grown in 40 mg/L P exhibited the lowest average biomass (12.69 g/plant) (Chrysarginis et al., 2016). A separate study conducted by Nell (et al., 2009), evaluated the biomass production of garden sage (Salvia officianalis L.) in response to phosphorus supply. Nell (et al., 2009) reported leaf biomass was significantly increased by 1.2-fold in the full rate (136 mg KH$_2$PO$_4$/L) versus the half rate (68 mg KH$_2$PO$_4$/L).

A proper fertility program is the key to producing healthy and uniform tobacco transplants. While nitrogen is the most critical nutrient, others such as phosphorus, play a large role even if they are not required in large concentrations. For organic flue-cured tobacco
growers, managing a fertility program can be very challenging due to the many different factors that impact nutrient release and availability with organic sources. In general, organic fertilizers do not have large quantities of N in a plant available form of nitrogen and are typically high in phosphorus (P) concentration and low in potassium (K). To account for this lack in potassium, growers have the option to apply supplemental sulfate of potash (0-0-52), a water soluble form of potassium.

**Organic Nitrogen Fertilizer Sources**

Conventionally grown tobacco is fertilized with synthetic, inorganic fertilizers that are available for plant uptake, or become quickly available through hydrolysis reactions in the soil (Havlin et al. 2005). Nitrogen assimilated by plants may be in several forms, but nitrate (NO$_3^-$), ammonium (NH$_4^+$), and atmospheric N account for most N taken up in natural ecosystems and in agriculture (Franco and Munns, 1982). Nitrate and ammonium-N combined compile just 5% of the total amount of soil N, while the remaining 95% of the total soil N is organic and includes proteins, amino sugars, and nucleic acids. Generally speaking, organic fertilizers are low in N and potassium, yet high in phosphorus. Nitrogen, the most critical nutrient in tobacco transplant production, is often considered to be a limiting factor in the production healthy and uniform seedlings.

Providing sufficient soil nitrogen availability to reach maximum yield potential can be a challenge in organic culture (Hartz et al., 2010). This is a great concern amongst organic flue-cured tobacco producers as nitrogen impacts the yield and quality of a flue-cured tobacco crop more so than any other nutrient. To accommodate the high nitrogen demands that tobacco plants require, leguminous cover crops can be utilized to provide additional nitrogen to the soil. Cover
cropping is generally the most economical way to provide plant-available nitrogen in organic systems (Gaskell and Smith, 2007). Other ways soil nitrogen is increased is from the decomposition of plant residues and soil organic matter, both of which are regarded as organic amendments that decompose at a relatively slow rate (Hadas and Kautsky, 1994). However, for profitable crop production, farmers cannot rely on organic amendments as the sole source of available nitrogen, and need materials that release nitrogen at a greater rate for pre-planting or top fertilization (Hadas and Kautsky, 1994). There are different options available to growers that enable them to produce an organic tobacco crop with the sufficient amount of nitrogen. An application of manure-based compost is a common organic production practice, but the low nitrogen mineralization rate typical of compost limits its nitrogen contribution (Hartz et al. 2010). Hartz et al. (2000) reported that most compost mineralized less than 10% of initial nitrogen content in the four to six months following soil incorporation. A high compost application rate can make a significant contribution to soil nitrogen availability, but in practice, application rate is constrained by cost and by the potential for water quality degradation from high phosphorus loading (Sharpley et al., 1994). An alternative option available to organic flue-cured tobacco producers is the use of dry organic fertilizers such as fishery waste, feather meal, and seabird guano, all of which high a high nitrogen content (>10% of dry weight) and relatively rapid nitrogen mineralization (Hartz et al. 2010). In contrast to manure-based compost, dry organic fertilizers mineralize 60% to 80% of nitrogen within four to eight weeks after application to agricultural soils (Hadas and Kautsky, 1994; Hadas and Rosenberg, 1992; Hartz and Johnstone, 2006). As previously mentioned, a popular dry organic fertilizer is seabird guano. Guano, an organic fertilizer, is a natural deposit of the excrement and remains of birds living along ocean coasts and feeding on fish (Hadas and Rosenberg, 1991). Guano has been an
important fertilizer before industrial fixing of nitrogen from the air was developed (Hadas and Rosenberg, 1991). Nitrogen in organic fertilizers is claimed to be water insoluble, and it may be slowly released with the decay of the fertilizer (Cooke, 1972). However, guano was found to be nitrified in soil at a considerably more rapid rate than other organic fertilizers (Owen et al., 1950). While guano is nitrogen rich, rapidly decomposable, and the most commonly used organic fertilizer, it is also an expensive material (Hadas and Kautsky, 1994). A considerably cheaper substitute for guano is feather meal. Feather meal, like guano, is also high in nitrogen content and is a by-product of poultry processing plants (Hadas and Kautsky, 1994). Ninety percent of feather dry weight consists of crude keratin protein, which, if processed by steam hydrolysis, can increase immediate release of nitrogen, with most occurring in the first five weeks of incubation while microbial hydrolysis increased nitrogen release during the 8-11 week period (Jong-Myung and Nelson, 1995). The conversion of organic nitrogen to an inorganic form is essential for enabling plant uptake. That said, the nitrogen cycle can be very complex and the forms of nitrogen are very dynamic.

**Fates of Nitrogen**

The potential sources of nitrogen in organic production are primarily composed of organic materials and occasionally small amounts of soluble nitrate or ammonium. Organic sources of nitrogen will go through a mineralization process in which soil microbes metabolize organic carbon and convert organic nitrogen into ammonium and a subsequent process that quickly oxidizes the NH$_4$$^+$ to NO$_3$$^-$ (Gaskell and Smith, 2007). Mineralization occurs through the activity of heterotrophic microorganisms that require organic carbon (C) for energy (Havlin et al., 2014). The mineralization of nitrogen to NH$_4$$^+$ occurs through two reactions, aminization and ammonification. Aminization converts proteins in residues to amino acids, amines and urea,
which are all organic compounds that are further converted to inorganic NH$_4^+$ by ammonification (Havlin et al., 2014). Ammonification is the first step in mineralization of organic nitrogen and is defined as the biological conversion of organic nitrogen to ammonium nitrogen (Reddy et al., 1984). The NH$_4^+$ that is produced through ammonification is subject to several fates, including nitrification, plant uptake, NH$_4^+$ fixation, volatilization, or immobilization (Havlin et al., 2014).

Several factors that influence mineralization rates include, but are not limited to, soil pH, soil moisture, and soil temperature. Specifically, all three of the previously mentioned factors influence aerobic and anaerobic microbial bacteria. In general, as pH increases, mineralization rates will also increase due to an increase in microbial activity. An argument can be made that soil moisture and temperature influence mineralization more so than other factors. Maximum aerobic activity and nitrogen mineralization occur between 50 and 70% water-filled pore space, while optimum soil temperature for microbial activity ranges between 25 and 35°C (Havlin et al., 2014). Nitrogen mineralization is always coupled with immobilization in the soil environment, and a complex set of factors determines whether nitrogen is released for plant uptake or remains immobilized by soil microbes (Jarvis et al., 1996).

Nitrogen immobilization is the conversion of inorganic nitrogen back to organic nitrogen (Havlin et al., 2014). If decomposing residues contain low nitrogen, microorganisms will immobilize NH$_4^+$ and NO$_3^-$ in the soil solution (Havlin et al., 2014). One of the main factors that influence immobilization is the ratio of carbon to nitrogen (C:N ratio). Microbes need nitrogen in a C:N ratio of roughly 8:1; therefore, inorganic soil nitrogen is utilized by the rapidly growing population, reducing NH$_4^+$ and NO$_3^-$ to very low levels (Havlin et al., 2014). While this can potentially cause plants to become nitrogen deficient, most cropping systems are exposed to sufficient nitrogen to compensate for nitrogen immobilization by microbes and crop nitrogen
uptake (Havlin et al., 2014). C:N ratios of materials used in organic production prove to be valuable information because ratios in soil organic matter are relatively constant, but if a material is added having a high C:N ratio (above 20-30) net nitrogen immobilization will occur due to increased microbial activity (Prasad and Power, 1997). If nitrogen immobilization does not occur, then the nitrification process will generally ensue.

It is known that $\text{NO}_3^-$ is produced through the oxidation of $\text{NH}_4^+$. This is a two-step process where ammonium is first converted to nitrite ($\text{NO}_2^-$) and then to nitrate. There are two groups of bacteria that help facilitate this process. The first group of bacteria, ammonia-oxidizing bacteria (AOB), converts ammonia to $\text{NO}_2^-$; then, the second group, nitrite-oxidizing bacteria (NOB), further oxidizes the intermediate product to nitrite (Jianlong and Ying, 2003). Nitrite generally does not accumulate in soils, which is fortunate since nitrite is toxic to plant roots (Havlin et al., 2014). In a greenhouse float bed system, where soilless media is used, populations of beneficial organisms, such as nitrifying bacteria, are low or nonexistent (Pearce et al., 1998). Furthermore, nitrifying bacteria are known to be relatively scarce in peat, a major component in soilless media (Herlihy, 1972). Despite low initial nitrifier populations, in most soilless mixes, several studies show that the nitrification rate increases as a crop is grown in the media (Elliot, 1972; Lang and Elliot, 1991). According to Elliot (1986) and Lang and Elliot (1991), nitrification peaked at 4 to 6 weeks of cropping and then declined. Such patterns could influence the availability of nitrogen to plants and the accumulation of toxic components (Pearce et al., 1998). High fertilizer rates or a high proportion of $\text{NH}_4^+$ nitrogen may also decrease nitrification activity in the soilless media (Lang and Elliot, 1991). It is already known that having nitrogen in the $\text{NH}_4^+$ form is the first requirement for nitrification to occur. In addition to $\text{NH}_4^+$ concentration, soil pH, soil aeration, soil moisture, and soil temperature all impact nitrification.
Although the oxidation of NH$_4^+$ to NO$_3^-$ takes place over a wide range in pH (5-9), optimum pH is roughly 8 (Havlin et al., 2014). Nitrifying bacteria need an adequate supply of calcium and micronutrients (Havlin et al., 2014). In addition to proper soil pH, adequate soil moisture is also required for optimal nitrification rates. Aerobic nitrifying bacteria will not produce NO$_3^-$ in the absence of oxygen (Havlin et al., 2014). Soils that are coarse textured or possess good structure facilitate rapid gas exchange and ensure adequate supply oxygen for nitrifying bacteria (Havlin et al., 2014). Soil moisture and soil aeration of closely related when comparing their impacts on nitrification. Nitrification declines as soil moisture exceeds field capacity or nears air dryness (Havlin et al., 2014). In general, the more moisture that is present in a soil system, the less oxygen there is available for nitrifying bacteria. Soil temperature also plays a major role in the nitrification process. Although nitrification occurs over a wide temperature range, optimum soil temperature is 25-35°C (Havlin et al., 2014). Before nitrate is produced, through nitrification, organic fertilizers must first be converted to ammonium through the mineralization process. Nitrification is essential to produce plant-preferable available form nitrogen, but in anaerobic environments, such as a greenhouse float bed system, denitrification can occur.

Denitrification is the biological reduction of nitrate to nitrogen gas by facultative heterotrophic bacteria. Large populations of denitrifying microorganisms exist, but the most common are the bacteria Pseudomonas, Bacillus, and Paracoccus, and several autotrophs (Havlin et al., 2014). Denitrification potential is high in most field soils, but suitable conditions must be present to cause a shift from aerobic respiration to a denitrifying metabolism involving nitrate as an electron acceptor in the absence of conditions between years, between seasons, and within a given field (Havlin et al., 2014). The primary pathways of gaseous nitrogen losses are by denitrification and ammonia volatilization (Havlin et al., 2014). Some anaerobic organisms
obtain their oxygen from nitrite and nitrate, with the accompanying release of nitrogen gas and nitrous oxide (Havin et al., 2014). Since nitrogen gas has low water solubility, it escapes into the atmosphere as gas bubbles. The potential for denitrification and the magnitude of nitrogen loss is dependent on three things major things: whether the surface environment is waterlogged or anaerobic, whether NO$_3^-$ and to a lesser extent NO$_2^-$ present in the anaerobic zone, and whether or not the surface soil contains an ample supply of decomposable or soluble carbon (Havlin et al., 2014). The occurrence of these three factors generally leads to a high denitrification potential and nitrogen loss.

Soil moisture content is critical to denitrification because of its effect on aeration (Havlin et al., 2014). In waterlogged environments, oxygen is excluded and anaerobic conditions exist. Generally, when soil water-filled pore space exceeds 60%, aerobic activity declines, while anaerobic microbial activity increases; however, appreciable nitrogen loss generally occurs at >80% water-filled pore space (Havlin et al., 2014). Denitrification accelerates under low oxygen diffusion in soil and a high microbial respiratory demand (high carbon source) (Havlin et al., 2014). When oxygen content of well-aerated soils, roughly 16-18%, declines to 8-10% oxygen, denitrification is optimized (Havlin et al., 2014). By definition, nitrate nitrogen must be present for denitrification to occur, and high nitrate increases denitrification potential (Havlin et al., 2014). However, nitrate will be readily denitrified if anaerobic conditions occur after the initial fertilizer nitrogen application (Havlin et al., 2014). According to Hanaki et al. (1990), denitrification may occur simultaneously with nitrification in a combined system at low dissolved oxygen while it is unlikely to happen at sufficient dissolved oxygen. Aeration status of the media in a tobacco float system may also influence the rates of nitrogen transformation (Pearce et al., 1998).
Influence of Aeration on Nitrogen Availability

In general, flooded soils and sediments are characterized by the absence of oxygen compared to well-drained soils (Reddy et al., 1984). Nitrogen in flooded soils and water columns occurs in inorganic and organic forms with the organic form predominating (Reddy et al., 1984). As previously mentioned in this chapter, inorganic nitrogen includes ammonium, nitrate and nitrite. Ammonium is the predominant form of inorganic N in the sediments and is mainly derived through mineralization of organic N (Reddy et al., 1984). The gaseous forms of N that occur in flooded soils and sediments include ammonia, dinitrogen, and nitrous oxide. It was discussed earlier in this chapter that ammonium is formed from organic N sources through ammonification, which is the first step in mineralization. Under anaerobic soil conditions, ammonium N accumulation occurs because of complete suppression of nitrification in soil zones which are devoid of oxygen (Reddy et al., 1984). If oxygen is not present after the formation of ammonium nitrogen, nitrification will be severely reduced. This reduction in nitrate can be attributed to the lack of microbial populations necessary for nitrification to occur (Pearce, 2016).

Tobacco is particularly susceptible to injury caused by anaerobic conditions (Nagao, 1972). Harris and van Bavel (1957) investigated the growth of tobacco root systems and concluded that excessive carbon dioxide in the root zone was the major contributing factor to plant injury. Aerobic respiration in plant roots is necessary for growth, mineral uptake, and indirectly, may affect water uptake (Lemon and Wiegand, 1961). Previous studies have demonstrated that low-oxygen partial pressures retard root growth by inhibiting respiration, reducing root branch formation, and even initiating root death (Hoagland and Broyer, 1936; Leonard and Pinckard, 1946; Ruff et al., 1987). Excess water in the root environment of plants can be injurious because it blocks the transfer of oxygen between the soil and the atmosphere.
Since air into and out of the stagnant water surface of the tobacco float system occurs very slowly, water roots generally develop in an anaerobic environment, which affects their appearance and function (Caruso et al., 2000). For example, Harris and Bavel (1957) investigated the nutrient uptake by tobacco plants and concluded that an oxygen partial pressure lower than 10% reduced nutrient solution uptake. This could be attributed to the growth of water roots being restricted under conditions of poor aeration, since the rapidly metabolizing zone of cell division near the root tip is the first to be affected by suboptimal aeration conditions (Caruso et al., 2000). Caruso et al. (2000) conducted a study comparing a control tank with tobacco transplant roots versus a tank where nitrogen gas was added to the solution where the roots were located. Caruso et al. (2000) visually observed that the near anaerobic conditions in the nitrogen gas treated float tanks reduced the shoot growth compared to the plants grown in the control tank. In addition, the tobacco plants grown in the nitrogen gas float tank had a much smaller shoot with reduced leaf sizes. Undoubtedly, the growth of tobacco seedlings can be negatively influenced by anaerobic conditions in the float system (Caruso et al., 2000).

Nitrification is one process that can be impacted by aeration. As previously discussed, nitrification is the conversion of ammonium nitrogen to nitrate nitrogen. This process can be slowed when there is a high presence of organic material. Hanaki et al. (1990) reported that high organic loading results in lower nitrification percentage because of (1) loss of ammonia for assimilation of heterotrophs and (2) the inhibitory effect of crowded cells of heterotrophs on ammonia oxidation. Furthermore, Kiff (1972) indicated that a low dissolved oxygen level may strengthen the effects of organic loading. Park et al. (2014) and de Graaf et al. (2010) indicated that 1.5-2.5 mg/L dissolved oxygen is beneficial for partial nitrification, whereas an excessively lower dissolved oxygen concentration (≤0.5 mg/L) may reduce nitrification rates. During this
conversion, there is an intermediate form of nitrogen produced, nitrite. Nitrite generally does not accumulate in soils, which is fortunate since nitrite is toxic to plant roots (Havlin et al., 2014). It was proposed by Ruiz et al. (2003) that dissolved oxygen is an effective controlling parameter for the stable maintenance of nitrite accumulation. Ruiz et al. (2003) continued by reporting that dissolved oxygen values higher than 1.7 mg/L prevented nitrite accumulation. A study conducted by Hanaki et al. (1990) concluded that unaffected ammonia oxidation and strongly inhibited nitrite oxidation at low dissolved oxygen resulted in accumulation of nitrite as an intermediate. Nitrite levels reached almost as high as 60 mg/L in a low dissolved oxygen system (0.5 mg/L), whereas the sufficient dissolved oxygen system (≥6.0 mg/L) maintained a low nitrite level (Hanaki et al. 1990). The high growth yield of ammonia oxidizers at the low dissolved oxygen level mentioned above may contribute to the nitrite accumulation through different rates of ammonia oxidation and nitrite oxidation (Hanaki et al., 1990).

**Impact of Bicarbonates in a Float Bed System**

Detrimental effects of bicarbonates on plant growth in soilless media and/or solution culture have been demonstrated for many plant species, including tomato (*Lycopersicon esculentum*) (Bailey and Hammer, 1986), petunia (*Petunia hybrida*) (Bailey and Hammer, 1986), soybean (*Glycine max*) (Dofing et al., 1989), amongst several other species (Rideout et al., 1995). High bicarbonate levels can interfere with the absorption of essential elements by plant roots and also have an undesirable influence upon nutrient solubility by altering the pH of the growth media (Lunt, 1956). Bicarbonate toxicity symptoms include severe chlorosis and stunted growth, but plants are usually not killed (Rideout et al., 1995). Rideout et al. (1995) observed that tobacco seedlings initially showed symptoms of bicarbonate injury approximately four weeks after seeding and that the onset of damage symptoms approximately coincides with the
emergence of roots into the nutrient solution. This would be the expected timing of injury because the buffering capacity of medium would not protect roots extending into the nutrient solution (Rideout et al., 1995). Previous research indicates that these symptoms are probably not the result of direct toxic influences of bicarbonate ions but rather diminished uptake and utilization of other essential elements (Kramer and Peterson, 1990). This is believed to result from chemical reactions between bicarbonate ions and nutrient ions both outside and within plant tissues (Lunt et al., 1956).

A high presence of bicarbonate and carbonate ions will often time produce high alkalinity levels, as alkalinity results primarily from the concentration of dissolved carbonate and bicarbonate ions (Lunt, 1956). Kramer and Peterson (1990) studied the effect of five alkalinity levels (0, 100, 250, 500, 100 mg HCO₃⁻/L) of irrigation water for *Chrysanthemum morifolium* in a greenhouse study. Their results showed applications of water containing 500 mg HCO₃⁻/L or more bicarbonate decreased plant height, fresh weight, and dry weight. These results with flue-cured tobacco would likely result in poor seedlings that potentially could not survive field conditions. A study conducted by Coggins (1992) investigated the effects of bicarbonate presence in float water on tobacco seedling development and growth. The experiment consisted of treatments with four different rates of bicarbonate (0, 4, 8, and 12 meq/L) in float water for direct seeded flue-cured tobacco. Coggins (1992) collected solution samples 28 and 48 days after seeding (DAS). Bicarbonate levels in the float water at 28 days averaged 0.6, 1.3, 5.3, and 9.5 meq HCO₃⁻/L for the 0, 4, 8, and 12 HCO₃⁻/L treatments, respectively (Coggins, 1992). The results from this same study showed that while seedling emergence was not impacted by bicarbonate presence, stem length, total seedling production and usable seedlings were all reduced by the presence of bicarbonate ions. Coggins (1992) reported that stem length decreased
6.3 cm to 5.3, 2.0 and 0.0 cm as the bicarbonate added increased from 0, 4, 8 and 12 meq HCO₃⁻/L. In addition, there was nearly a 50 percent reduction in seedlings as bicarbonate levels increased from 0 or 4 to 8 meq HCO₃⁻/L and the seedlings died at the 12 meq HCO₃⁻/L rate (Coggins, 1992). Total usable seedlings decreased remarkably at bicarbonate levels above the 4 meq HCO₃⁻/L rate, according to Coggins (1992) results. High alkalinity and bicarbonate levels in a float bed system can be reduced, however.

The practice of injecting acid to irrigation water has been recommended to diminish the undesirable influences of high pH and alkalinity levels which are found in some irrigation water (Kramer and Peterson, 1990). This practice has resulted in an improvement in the growth and quality of greenhouse crops (Hageman and Hartman, 1941). In the study conducted by Kramer and Peterson (1990), acidification of the irrigation water was beneficial to the growth of Chrysanthemum morifolium. Their results showed acidification of irrigation water was clearly beneficial when alkalinity levels exceeded 500 mg HCO₃⁻/L. Furthermore, they concluded that the addition of acid to irrigation water resulted in a significant increase in plant dry weight and plant height when alkalinity levels were ≤ 500 mg HCO₃⁻/L. The type of acid used to acidify water should also be taken into consideration. Kramer and Peterson (1990) found differences in plant growth due to the type of acid used. There are a number of acids that are available to growers, including nitric, phosphoric, hydrochloric, and sulfuric (Reynolds, 2000). However, these acids vary widely in concentration, particularly sulfuric acid (Reynolds, 2000). According to Kramer and Peterson (1990), sulfuric acid amended irrigation water resulted in a significant increase in chrysanthemum height and fresh weight as compared to plants treated with phosphoric and nitric acid amended water. Overall, results indicate that sulfuric acid was the most effective acidifying agent among the acid tested for improving chrysanthemum growth in
situations where the irrigation water pH and alkalinity require modification (Kramer and Peterson, 1990).

Overall, acidification appears to be the single most important factor in obtaining consistently acceptable plant growth with high HCO$_3^-$ water (Rideout et al., 1995). According to Rideout et al. (1995), acidification should be used as the primary method of preventing HCO$_3^-$ damage in tobacco seedling production. However, since the buffering of solution pH is lost, and pH decreases rapidly when HCO$_3^-$ is totally neutralized, extreme care must be taken to prevent over-acidification of the solution (Rideout et al., 1995). For this reason, leaving up to 1.5 meq L$^{-1}$ of HCO$_3^-$ in solution to provide buffering is desirable (Rideout et al., 1995). According to data presented by Rideout et al. (1995), significant plant injury will not occur at 1.5 meq L$^{-1}$ of HCO$_3^-$.

It is evident that organic seedling producers face many challenges that conventional seedling producers do not encounter. Nutrient availability is of great concern with many organic fertility sources. Nutrient release from these organic sources may be rapidly advanced if aeration is added to the float water environment. This research was conducted to evaluate the impact aeration can have on nutrient release, specifically nitrogen.

**MATERIALS AND METHODS**

**Experimental Design and Greenhouse Procedures**

From 2016-2017, a study was conducted to evaluate organic flue-cured tobacco seedling production in a float-bed system. The experiment was conducted at the Central Crops Research Station in Clayton, North Carolina in a greenhouse located on the station. The greenhouse structure is typical of tobacco greenhouses used in North Carolina, having a plastic cover, side
curtains, heaters, and two fans to keep air circulating. The objective of this study was to compare a conventional complete fertilizer source (16-5-15) to multiple organic fertilizer sources consisting of sodium nitrate (16-0-0), pelletized Peruvian seabird guano (12-11-2), or a combination of both, and to quantify nutrient availability and seedling growth. In total, 5 fertilizer treatments were developed and evaluated in aerated and non-aerated float beds.

To provide adequate growing space and to separate treatments, 30 mini beds were constructed inside of the greenhouse with each mini bed designed to hold six 288 cell expanded polystyrene (EPS) trays. Each tray had the center 100 cells of the total 288 cells marked off in order to collect data from this region throughout the study. These trays were seeded with organic soilless media on June 4, 2016 and November 9, 2016 with variety K 326 and then floated in the proper bed. All beds were filled with water from a deep well to a depth of 12.7 cm. A Hydor bubble maker, which acted as an aerator, was placed in the center of each mini bed to provide additional oxygen to the environment. All fertilizers were applied directly into the solution of each individual bed 9 and 21 Days After Seeding (DAS). When guano was included in a treatment, it was ground to pass through a 1 mm sieve in a food processor to increase surface area, thus increasing solubility. Prior to applying the fertilizer, all components of each fertility program were placed in a sterile, 473.6 mL hard-plastic bottle designated for each individual treatment. Hot tap water (50 ºC) was then added to each bottle and then each bottle was shaken for two minutes in an attempt dissolve the fertilizer. The dissolved components were transferred to the greenhouse and then evenly distributed in the appropriate mini bed. This process was repeated for the second fertilizer application.

Nitrogen, phosphorus (P) and potassium (K) nutrient rates targeted 125, 40, and 125 mg/L, respectively, for the complete 16-5-16 conventional fertility program, the seabird guano
plus sodium nitrate program, and seabird guano and sodium nitrate plus gypsum. When seabird guano was applied as the exclusive source of N, 125 mg/L of N and K were targeted. Targeting this rate of N for these two treatments resulted in 115 mg P/L. Where gypsum is included in the fertility program, 50 mg Ca/L was targeted.

Throughout the study, nutrient availability was quantified to measure the differences among treatments. Every five days the water depth would be elevated to 12.7 cm in each bed and then a solution sample was collected from all 21 mini beds. Solution samples were collected in a sterile hard-plastic bottle and were immediately taken to North Carolina Department of Agriculture and Consumer Services-Agronomic Division for analysis. In addition to collecting solution samples, dissolved oxygen was also measured every five days using a YSI Professional Plus dissolved oxygen meter. Two readings were collected from each bed: one from the edge of the bed and one from the center. The YSI Professional plus also recorded the temperature of the water in each mini bed. Further data collected included percent spiral root and soluble salts injury, which was collected 15 and 20 DAS, while percent germination was solely collected at 15 DAS. A percentage for each of the previous data categories was obtained by examining the middle 100 cells from each individual tray. Each percentage from each individual tray was then added and then divided by six, the number of trays in each mini bed. Furthermore, a media sample was collected 20 DAS from within the center 100 cells that were marked off and used for data collection. Fifteen media samples were collected from each tray for a total of 75 samples per treatment. A second media sample was collected at the conclusion of each study and followed the same procedures. Media samples, along with tissue samples, were collected and immediately taken to North Carolina Department of Agriculture and Consumer Services Agronomic Division for analysis. In addition to collecting media samples at the conclusion of the study, ten whole
tissue samples were also collected from each bed, detached from the soilless media, and then measured for stem height and diameter. Stem height was measured from the base of the stem to the base of the bud, while stem diameter was measured at the base of the stem using a caliper. In addition, at the conclusion of the study the total plants produced was quantified and then the amount of usable transplants was recorded. Total plants only included plants that were within the 100 cell barrier that was marked at the beginning of the study. Usable transplants were defined as transplants that would likely be healthy enough to survive field conditions once transplanted.

**Analytical Procedures**

**Solution Samples**

*Nitrogen.* The inorganic-N fraction concentrations include NO$_3^-$, nitrite (NO$_2^-$), and NH$_4^+$ nitrogen. The organic N fraction concentration includes urea. Nitrate is determined on ~10 mL homogenized sample, filtered using acid washed filter paper (Laboratory Filtration Group, Houston, TX) by nitrate-hydrazine reduction (Kempers 1988; Skalar Analytical 1995a); Ammonium is determined by a modified Berthelot reaction (adapted from Krom 1980; Skalar Analytical 1995b); and urea concentration is determined with the diacetyl monoxime thiosemicarbazide colorimetric method (Sullivan and Havlin, 1991; Skalar Analytical 1995, Issue 6) with an auto-flow spectrophotometric analyzer (San++ Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands).

*pH.* The pH values are measured directly on homogenized samples. The pH is measured using a hydrogen electrode (Orion 920A; Thermo Fisher Scientific; Beverly, MA) (Eaton et al., 2005).

*Bicarbonate.* Bicarbonate concentrations are measured by titration using phenolphthalein and methyl orange as endpoint indicators (AOAC, 1990).
Media Samples

Samples were analyzed as received by the North Carolina Department of Agriculture and Consumer Services – Agronomic Division. pH is measured on a 1:1 v/v ratio of fresh sample to DI water following a 60 minute equilibration. The saturated media extract is obtained by adding DI water to a fresh ~400 mL sample until it reaches a saturation point. The sample is allowed to equilibrate for 60 minutes, and then the liquid is extracted from the sample by vacuum filtration (Whatman 1 Qualitative Circles, 110 mm).

Nitrogen. Inorganic-nitrogen fraction concentrations include nitrate plus nitrite and ammonium nitrogen. Organic nitrogen fraction concentration includes urea. Nitrate nitrogen is determined on the saturated media extract, (~10 mL) by nitrate-hydrazine reduction (Kempers 1988; Skalar Analytical 1995a); Ammonium nitrogen is determined by a modified Berthelot reaction (adapted from Krom 1980; Skalar Analytical 1995b); and urea concentration is determined with the diacetyl monoxime thiosemicarbazide colorimetric method (Sullivan and Havlin, 1991; Skalar Analytical 1995, Issue 6) with an auto-flow spectrophotometric analyzer (San++ Segmented Flow Auto-Analyzer, Skalar Instruments; Breda, The Netherlands).

pH. The pH values are measured on a 1:1 v/v ratio of fresh sample to DI water following a 60 minute equilibration. The pH is measured using a hydrogen electrode (Orion 920A; Thermo Fisher Scientific; Beverly, MA) (Eaton et. al., 2005).

RESULTS AND DISCUSSION

Solution Analysis

Bicarbonates. Aeration reduced bicarbonate concentrations in the float bed solution. Bicarbonate presence in environment 1 and 2 (Figure 1) in the float bed solution is greatest in the
two treatments that contain seabird guano as the sole source of nitrogen in non-aerated beds. When these same two treatments are placed in an aerated bed, there is a significant reduction in bicarbonate presence (Figure 1). Bicarbonate concentrations in both environments peaked 25 days after seeding and decreased throughout the duration of the study (Figure 1). It must be noted that bicarbonate presence did not impact total or usable transplants (Table 1), even though concentrations were observed greater than 14 meq/L in environment two 25 days after seeding. Results from Environment 1 where seabird guano was coupled with gypsum and 0-0-52 show aeration reduced bicarbonate concentration from 11 meq HCO\textsubscript{3}^{-}/L to 1.6 meq HCO\textsubscript{3}^{-}/L in solution samples collected 30 DAS (Figure 1). Solution samples collected 40 DAS from the same treatments in Environment 2 reported a decrease in HCO\textsubscript{3}^{-} concentration from 9.1 meq HCO\textsubscript{3}^{-}/L to 2.2 meq HCO\textsubscript{3}^{-}/L (Figure 1). When sodium nitrate is added to the fertility program with seabird guano, bicarbonate concentration in non-aerated float water never exceeds 3.2 meq HCO\textsubscript{3}^{-}/L in Environment 1 and 4.5 meq HCO\textsubscript{3}^{-}/L in Environment 2 (Figure 1). This further indicates reducing seabird guano and adding sodium nitrate to the fertility program can reduce bicarbonate concentrations, even when the float water solution is not aerated.

Bicarbonate concentration can raise the pH of solution water and therefore reduce nutrient uptake by plants. Buffering capacity could explain why bicarbonate toxicity symptoms were not observed in this study. Seabird guano, which is high in NH\textsubscript{4}^{+} concentration, releases hydrogen (H\textsuperscript{+}) ions into the float water solution as it oxidizes to form NO\textsubscript{3}-. The additional H\textsuperscript{+} ions released into solution acts an acid and buffered against the high concentrations of HCO\textsubscript{3}^{-}. In this scenario, the H\textsuperscript{+} ions kept the pH at a value where nutrient uptake was not diminished to a point where plant growth was reduced. Previous research conducted on L. microphyllum has
shown where plants grown in pH 8.0 produced 50% of the biomass compared to *L. microphyllum* grown in pH’s of 5.5 and 6.5 (Soti et al., 2015).

**Dissolved Oxygen.** Treatments that were applied to aerated beds contained a significantly higher dissolved oxygen concentration in the solution than did the same fertility programs placed into non-aerated float beds. This observation was consistent in environments 1 and 2 (Figure 2). A decrease in NH$_4^+$ and HCO$_3^-$ concentration and increase in NO$_3^-$ concentration was observed in treatments where dissolved oxygen concentration was greatest (Figures 3 & 4). This observation suggests that additional oxygen in the float water solution might increase nitrification rates. A decrease in HCO$_3^-$ could also be attributed to increased nitrification rates, as it is possible that as NH$_4^+$ nitrifies, H$^+$ is released into solution. The addition of H$^+$ ions reacts with HCO$_3^-$ to produce CO$_2$ and H$_2$O.

**Nitrate.** Data from environment one and environments two (Figure 3) demonstrate that aeration significantly improved the availability of NO$_3^-$ in treatments that contained only seabird guano as the source of N. Initially, in each environment, there was a negligible amount of NO$_3^-$ present in the float water solution of these two treatments, regardless of whether or not they were applied in an aerated or non-aerated float bed. Nitrate levels from both Environments where seabird guano was the exclusive N source did not exceed 5 mg/L (Figure 3). However, beginning 25 and 40 days after seeding in environment one and environment two, respectively, NO$_3^-$ begins to increase in the seabird guano based treatment (Figure 3). Comparing NO$_3^-$ levels among these two treatments in aerated versus non-aerated beds shows significantly higher amounts of NO$_3^-$ present in the aerated float bed (Figure 3). It is possible that this occurs because additional aeration could increase nitrification rates. Havlin (et al., 2014) reports that an adequate supply of oxygen must be available for nitrifying bacteria to oxidize NH$_4^+$ to NO$_3^-$. This theory is
furthered by decreasing amounts of NH$_4^+$ seen across both environments in the two treatments where seabird guano is used as the sole source of N.

Environment two (Figure 3) shows NO$_3^-$ concentrations in the 16-5-16 conventional fertilizer treatment to be much lower than the same treatment in environment one (Figure 3), regardless of whether or not this treatment was placed into an aerated or non-aerated float bed. It must be noted that this impacted the inorganic nitrogen concentration for this treatment.

*Ammonium.* In both environment one and environment two (Figure 4), NH$_4^+$ concentration in the float bed solution was highest in fertility programs that utilized seabird guano as the single nitrogen source. However, comparing these two treatments once placed in an aerated and non-aerated bed shows that aeration reduces the amount of NH$_4^+$ present in the float bed solution (Figure 4). Furthermore, while the NH$_4^+$ concentration in the aerated seabird guano float bed decreased in the latter half of the study in both environments, there was an increase in NO$_3^-$ during the same period of time (Figures 3 & 4). This trend was observed in both environments, and further suggests that nitrification rates could have been increased by aeration.

*Inorganic Nitrogen.* Inorganic nitrogen includes the combination of ammonium nitrogen and nitrate nitrogen. Environment one and environment two (Figure 5) follow similar trends for inorganic nitrogen present in the float bed solution. It does not appear that aeration plays a significant role in increasing inorganic nitrogen concentration at any point in either environment. Data from environment two shows much lower inorganic nitrogen concentrations in the 16-5-16 treatment compared to others in this study, regardless of whether this treatment was placed in an aerated or non-aerated bed (Figure 5).
**pH.** Typically, pH has an effect on nutrient release and availability, amongst other factors, such as bacteria. In environments one and environment two (Figure 6), pH fluctuates throughout the entirety of the study, but tends to increase after each fertilizer application. After the final application 21 days after seeding, pH in both environments decreases throughout the remainder of the study. This correlates with a decrease in HCO$_3^-$ concentration. It is possible that pH decreases because H$^+$ ions are being released into solution as NH$_4^+$ is oxidized to NO$_3^-$. Additional H$^+$ ions in the float water solution would essentially act as an acid and therefore, lower the solution pH. Adding fresh water from a deep well every 5 days could also lower the pH. The well water pH being lower than the float water solution would cause a gradual decrease. Overall, in both environments, it does not appear where aeration impacted the pH of these treatments, except the 16-5-16 conventional fertility treatment in environment two (Figure 6).

**Urea.** Urea concentration varied in both environments (Figure 7). Results from both environments show treatments containing seabird guano as the sole source of nitrogen applied in aerated float water solution having the greatest concentration of urea (Figure 7). This release of urea might be attributed to additional oxygen and movement in the float water solution. This additional activity could increase the organic N being released from the seabird guano. Once the organic N is released, it can undergo mineralization and nitrification to produce a plant available form of N.

Urea concentration could explain an increase in bicarbonate presence that was observed in both environments (Figure 1). Greatest concentrations of urea and bicarbonate was present 25 DAS. Urease is a nickel-dependent enzyme, present in various plants and microorganisms that catalyzes the hydrolysis of urea to ammonia and carbon dioxide, which causes an abrupt increase of pH (Karplus et al., 1997; Zerner, 1991). An increase in CO$_2$ could lead to H$^+$ and an
additional oxygen ion binding, thus creating HCO$_3^-$ This would explain an abrupt increase in pH.

**Seedling Measurements**

Data collected on the transplants occurred after each tray had been clipped a minimum of five times. Clipping a minimum of five times allows smaller plants to catch the larger plants, increases stem diameter, and decreases stem height (Fisher and Vann, 2017). All of these factors can increase the quality of tobacco transplants.

*Total Plants.* Little differences were observed in respect to total transplants produced in this study (Table 1). When comparing each of the five treatments in aerated and non-aerated beds, there does not appear to be any trends that would leave one to believe that aeration impacted total plants produced. Numerically, and regardless of the aeration factor, the treatment that yielded the highest number of plants produced was when 16-0-0 and seabird guano was combined with sulfate of potash (0-0-52) and gypsum.

*Usable Plants.* All treatments produced a high percentage of usable transplants (Table 1). Numerically, the treatment that yielded the highest number of plants produced was when 16-0-0 and seabird guano was combined with sulfate of potash (0-0-52) and gypsum. A higher number of usable transplants in this treatment could be due a more balanced ammonium to nitrate ratio. In terms of usable plants, this treatment significantly outperformed the conventional 16-5-16 fertilizer. However, the results of this study do not suggest that conventionally grown tobacco be produced using organic fertility sources. Aeration did not seem to increase the total number of usable plants in this study.
**Stem Height.** Stem heights were measured from the base of the stem to the base of the bud. There were several differences observed in plant height among treatments, but no general trends were observed (Table 1). The difference the shortest versus tallest stem, according to treatment, is 1.88 cm. This statistical difference is not likely to have an agronomic impact. In addition, aeration did not seem to impact the height of plants. It should be noted that stem height did not seem to decrease plant usability in any of these treatments.

**Stem Diameter.** Several differences were observed in stem height between treatments (Table 1); however, the difference in the widest stem versus the slimmest stem, according to treatment, is 0.71 mm. This slight difference is not likely to have an agronomic impact. The treatment which included sodium nitrate and seabird guano plus sulfate of potash and gypsum, in aerated and non-aerated float beds, had the largest stem diameter. This treatment also yielded the highest percentage of usable plants.

**Soilless Media Analysis**

Soilless media samples were collected at two different intervals. Data for each sampling interval was pooled across both environments.

**20 Days After Seeding.** Media samples collected 20 days after seeding did not show any specific trends (Tables 2, 3, & 4). In addition, aeration did not seem to significantly impact nutrient concentration within the soilless media. As expected, soilless media from treatments that contained seabird guano as the exclusive source of nitrogen contained higher concentrations of ammonium and much lower nitrate levels (Tables 3 & 4). These results are likely due to much of the ammonium not having enough time to nitrify in order to produce higher amounts of nitrate nitrogen. Media pH was observed to be highest in treatments where seabird guano was the
exclusive source of N (Table 2). These treatments also had the greatest concentration of bicarbonate in solution (Figure 1). As evapotranspiration occurs, ions, including $\text{HCO}_3^-$, are accumulated in the soilless media. This increase in bicarbonate could explain the increase in media pH for these treatments.

**Conclusion of Study.** No obvious trends were observed in media samples collected on the final day of the study (Tables 5, 6, & 7). Media samples collected from float trays located in solution that had been fertilized with seabird guano showed higher amounts of nitrate in media collected at the conclusion of the study as opposed to 20 days after seeding (Table 5). This could be related to the seabird guano being exposed to environmental conditions for a prolonged period of time, thus allowing some of the ammonium to nitrify into nitrate.

pH of the soilless media for treatments containing seabird guano as the sole source of N decreased from the pH in media samples collected 20 DAS (Tables 6 & 7). The increase in nitrate and decrease in ammonium could be responsible for the pH decrease. As ammonium oxidizes to nitrate, a $\text{H}^+$ ion is released. Increasing $\text{H}^+$ decreases pH, especially when $\text{HCO}_3^-$ ions are not present to act as a buffer. Decreased $\text{HCO}_3^-$ concentrations were observed in solution, which possibly led to a decrease in the soilless media.

**CONCLUSION**

Organic tobacco production has experienced significant expansion over the past decade, but despite this expansion there has been very little information available to growers regarding greenhouse fertility management for transplant production. The purpose of this study was to evaluate different organic fertilizer programs in an effort to provide solutions for organic tobacco transplant producers. In addition, this study was designed to evaluate the same five fertility
programs in both an aerated and non-aerated float bed to determine the impact additional oxygen can have on nutrient release and availability. Maintaining an appropriate amount of nutrients, specifically nitrogen, is critical in the production of healthy, usable tobacco transplants. Furthermore, a balanced supply of ammonium and nitrate nitrogen is preferable. While there are concerns, such as high bicarbonate concentrations, with some of the treatments used in this study, none of these factors seemed to impact plant growth.

Research from 2016-2017 showed fertility programs that included seabird guano as the sole source of nitrogen can lead to very high bicarbonate concentrations, high ammonium levels, and decreased nitrate nitrogen in the float bed solution. However, results also show that aeration can play a significant role in nutrient availability and release. Bicarbonate concentrations in all treatments were reduced in the aerated float beds, when compared to non-aerated float beds. Furthermore, aeration appeared to increase nitrification rates in those organic fertility treatments where seabird guano was the exclusive source of nitrogen. With those treatments, data from this study shows that after a prolonged period of exposure ammonium concentration begins to decrease while nitrate nitrogen concentration begins to increase. This trend is observed in the non-aerated float bed solution, but is much more pronounced in the aerated float bed solution.

Ultimately, whether each of the treatments was applied into an aerated float bed solution or non-aerated, plant usability did not seem to be impacted. Each of the treatments produced a high percentage of usable plants. Having a fertility program that targets appropriate amounts of macro and micro nutrients is an important aspect of tobacco transplant production. Understanding the differences and challenges of organic transplant production, particularly nutrient availability and release, is an important step to producing high quality transplants. Further research is needed to evaluate the ability of an aeration system designed to provide
additional oxygen to a larger volume of water, similar to environments used by growers in North Carolina. Distributing the oxygen to all parts of a larger space could potentially be a challenge that organic growers could face, should they choose to incorporate aeration.
Literature Cited


Table 1. Total plants, usable plants, stem diameter, and stem heights as affected by fertility programa.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Plants</th>
<th>Usable Plants</th>
<th>Stem Diameter</th>
<th>Stem Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NA)</td>
<td>88 b</td>
<td>79 c</td>
<td>3.30 b</td>
<td>6.62 ab</td>
</tr>
<tr>
<td>16-5-16 (A)</td>
<td>90 ab</td>
<td>81 bc</td>
<td>3.42 ab</td>
<td>6.39 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>90 ab</td>
<td>79 c</td>
<td>2.76 c</td>
<td>5.62 bcd</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>91 ab</td>
<td>78 c</td>
<td>2.83 c</td>
<td>5.10 d</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>91 ab</td>
<td>85 ab</td>
<td>3.48 ab</td>
<td>6.27 abc</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>92 a</td>
<td>85 ab</td>
<td>3.54 a</td>
<td>6.88 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>93 a</td>
<td>86 a</td>
<td>3.43 ab</td>
<td>6.47 ab</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>93 a</td>
<td>87 a</td>
<td>3.42 ab</td>
<td>6.33 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>91 ab</td>
<td>79 c</td>
<td>2.84 c</td>
<td>6.08 a-d</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>90 ab</td>
<td>78 c</td>
<td>2.84 c</td>
<td>5.24 cd</td>
</tr>
</tbody>
</table>

aTreatment means followed by the same letter within the same column are not statistically different at the α=0.05 level. Data are pooled across two environments.

b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

c Non-Aerated float water solution
d Aerated float water solution
Table 2. Soilless media nutritional concentration 20 days after seeding\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Treatments\textsuperscript{b}</th>
<th>Urea</th>
<th>Soluble Salts</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NA\textsuperscript{c})</td>
<td>0.19 ab</td>
<td>174.17 e</td>
<td>5.30 e</td>
</tr>
<tr>
<td>16-5-16 (A\textsuperscript{d})</td>
<td>0.22 ab</td>
<td>171.83 e</td>
<td>5.39 de</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>0.17 b</td>
<td>172.17 e</td>
<td>5.72 bc</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>0.27 ab</td>
<td>173.00 e</td>
<td>5.96 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>0.31 ab</td>
<td>240.50 bc</td>
<td>5.60 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>0.12 b</td>
<td>215.50 cd</td>
<td>5.72 bc</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>0.15 b</td>
<td>279.17 a</td>
<td>5.55 cd</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>0.08 b</td>
<td>254.50 ab</td>
<td>5.65 c</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>0.47 a</td>
<td>189.50 de</td>
<td>5.90 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>0.32 ab</td>
<td>180.33 e</td>
<td>5.95 a</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter within the same column are not statistically different at the $\alpha=0.05$ significance level. Data are pooled across two environments.

\textsuperscript{b} 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

\textsuperscript{c} NA, Not-aerated float water solution

\textsuperscript{d} A, Aerated float water solution
Table 3. Soilless media nutritional concentration from Environment 1 collected 20 days after seeding\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Treatments\textsuperscript{b}</th>
<th>Inorganic nitrogen</th>
<th>Ammonium</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NA\textsuperscript{c})</td>
<td>128.67 b</td>
<td>22.97 c</td>
<td>105.80 b</td>
</tr>
<tr>
<td>16-5-16 (A\textsuperscript{d})</td>
<td>120.20 b</td>
<td>22.97 c</td>
<td>97.00 b</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>52.20 c</td>
<td>51.60 b</td>
<td>0.41 c</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>49.70 c</td>
<td>48.70 b</td>
<td>1.02 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>130.33 b</td>
<td>20.37 c</td>
<td>110.00 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>125.20 b</td>
<td>18.83 c</td>
<td>106.43 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>163.33 a</td>
<td>23.30 c</td>
<td>140.00 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>140.67 ab</td>
<td>22.07 c</td>
<td>118.47 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>65.40 c</td>
<td>63.50 a</td>
<td>1.89 c</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>51.23 c</td>
<td>49.87 b</td>
<td>1.32 c</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter within the same column are not statistically different at the $\alpha=0.05$ level.

\textsuperscript{b} 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

\textsuperscript{c} Non-Aerated float water solution

\textsuperscript{d} Aerated float water solution
Table 4. Soilless media nutritional concentration from Environment 2 collected 20 days after seeding.a.

<table>
<thead>
<tr>
<th>Treatments b</th>
<th>Inorganic nitrogen</th>
<th>Ammonium</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NA c)</td>
<td>17.67 b</td>
<td>17.27 b</td>
<td>0.42 c</td>
</tr>
<tr>
<td>16-5-16 (A d)</td>
<td>26.20 b</td>
<td>25.50 b</td>
<td>0.68 c</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>43.60 b</td>
<td>42.97 a</td>
<td>0.61 c</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>45.17 b</td>
<td>44.40 a</td>
<td>0.75 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>158.33 a</td>
<td>22.37 b</td>
<td>136.33 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>118.33 a</td>
<td>19.00 b</td>
<td>99.27 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>151.67 a</td>
<td>20.67 b</td>
<td>130.80 ab</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>126.33 a</td>
<td>20.73 b</td>
<td>105.47 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>39.37 b</td>
<td>38.87 a</td>
<td>0.57 c</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>44.17 b</td>
<td>43.73 a</td>
<td>0.44 c</td>
</tr>
</tbody>
</table>

aTreatment means followed by the same letter within the same column are not statistically different at the α=0.05 level.
b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
c Non-Aerated float water solution
d Aerated float water solution
Table 5. Soilless media nutritional concentration at the conclusion of the study\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Treatments\textsuperscript{b}</th>
<th>IN-N</th>
<th>Nitrate</th>
<th>Urea</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>--mS/cm--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-5-16 (NA\textsuperscript{c})</td>
<td>54.20 a</td>
<td>14.98 cd</td>
<td>0.11 bc</td>
<td>176.33 c</td>
</tr>
<tr>
<td>16-5-16 (A\textsuperscript{d})</td>
<td>47.27 ab</td>
<td>7.87 d</td>
<td>0.13 bc</td>
<td>166.50 c</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>40.35 ab</td>
<td>3.44 d</td>
<td>0.18 abc</td>
<td>242.00 b</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>35.68 ab</td>
<td>3.67 d</td>
<td>0.13 bc</td>
<td>246.67 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>50.50 a</td>
<td>46.73 a</td>
<td>0.26 ab</td>
<td>262.83 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>46.67 ab</td>
<td>44.05 ab</td>
<td>0.18 abc</td>
<td>272.67 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>40.26 ab</td>
<td>34.30 ab</td>
<td>0.13 bc</td>
<td>330.00 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>30.82 b</td>
<td>27.38 bc</td>
<td>0.21 ab</td>
<td>334.67 a</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>47.23 ab</td>
<td>3.42 d</td>
<td>0.04 c</td>
<td>268.17 b</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>42.20 ab</td>
<td>0.81 d</td>
<td>0.32 a</td>
<td>326.17 a</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Treatment means followed by the same letter within the same column are not statistically different at the $\alpha=0.05$ significance level. Data are pooled across two environments.

\textsuperscript{b}16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

\textsuperscript{c}NA, Not-aerated float water solution

\textsuperscript{d}A, Aerated float water solution
Table 6. Soilless media nutritional concentration from Environment 1 collected at the conclusion of the study.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ammonium</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NA)</td>
<td>52.83 a</td>
<td>5.39 bc</td>
</tr>
<tr>
<td>16-5-16 (A)</td>
<td>55.47 a</td>
<td>5.39 bc</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>41.53 b</td>
<td>5.03 c</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>45.43 ab</td>
<td>4.96 c</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>2.07 c</td>
<td>6.42 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>2.47 c</td>
<td>6.18 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>3.82 c</td>
<td>5.27 bc</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>2.24 c</td>
<td>5.87 ab</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>55.73 a</td>
<td>4.74 c</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>55.57 a</td>
<td>4.73 c</td>
</tr>
</tbody>
</table>

a Treatment means followed by the same letter within the same column are not statistically different at the α=0.05 level.

b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)

c Non-Aerated float water solution

d Aerated float water solution
Table 7. Soilless media nutritional concentration from Environment 2 collected at the conclusion of the studya.

<table>
<thead>
<tr>
<th>Treatmentsb</th>
<th>Ammonium</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-5-16 (NAc)</td>
<td>25.60 ab</td>
<td>4.86 e</td>
</tr>
<tr>
<td>16-5-16 (Ad)</td>
<td>23.30 ab</td>
<td>4.89 e</td>
</tr>
<tr>
<td>SG + 0-0-52 (NA)</td>
<td>32.23 a</td>
<td>5.29 bc</td>
</tr>
<tr>
<td>SG + 0-0-52 (A)</td>
<td>18.57 bc</td>
<td>5.57 b</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (NA)</td>
<td>5.45 d</td>
<td>6.79 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 (A)</td>
<td>2.72 d</td>
<td>6.82 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (NA)</td>
<td>8.06 cd</td>
<td>6.59 a</td>
</tr>
<tr>
<td>16-0-0 + SG + 0-0-52 + Gypsum (A)</td>
<td>4.61 d</td>
<td>6.61 a</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (NA)</td>
<td>31.90 a</td>
<td>5.09 cde</td>
</tr>
<tr>
<td>SG + 0-0-52 + Gypsum (A)</td>
<td>27.20 ab</td>
<td>5.18 cd</td>
</tr>
</tbody>
</table>

a Treatment means followed by the same letter within the same column are not statistically different at the α=0.05 level.
b 16-5-16 (SQM); 16-0-0 (SQM-Allganic); 0-0-52 (SQM-Allganic); SG, Peruvian Seabird Guano (Sunleaves 12-11-2)
c Non-Aerated float water solution
d Aerated float water solution
Table 8. P values for inorganic nitrogen, ammonium nitrogen, nitrate nitrogen, urea, soluble salts, and pH for media samples collected 20 days after seeding.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Environment (E)</th>
<th>Rep (Environment)</th>
<th>Treatment (Trt)</th>
<th>E x Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>0.0191</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>0.0002</td>
<td>---</td>
</tr>
<tr>
<td>Nitrate</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>pH</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Soluble Salts</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 9. P values for inorganic nitrogen, ammonium nitrogen, nitrate nitrogen, urea, soluble salts, and pH for media samples collected at the conclusion of the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Environment (E)</th>
<th>Rep (Environment)</th>
<th>Treatment (Trt)</th>
<th>E x Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>pH</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>Environment 1</td>
<td>---</td>
<td>---</td>
<td>0.001</td>
<td>---</td>
</tr>
<tr>
<td>Environment 2</td>
<td>---</td>
<td>---</td>
<td>&lt;0.0001</td>
<td>---</td>
</tr>
<tr>
<td>Inorganic Nitrogen</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Nitrate</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Soluble Salts</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Urea</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>
Table 10. Baseline solution and media nutrient concentrations collected from Environment 1 prior to seeding.

<table>
<thead>
<tr>
<th></th>
<th>Inorganic N</th>
<th>Urea</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Bicarbonate</th>
<th>Phosphorus</th>
<th>pH</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>9.00</td>
<td>0.35</td>
<td>3.26</td>
<td>5.74</td>
<td>--</td>
<td>0.13</td>
<td>4.56</td>
<td>106.80</td>
</tr>
<tr>
<td>Solution</td>
<td>3.96</td>
<td>0.54</td>
<td>0.55</td>
<td>3.41</td>
<td>0.30</td>
<td>0.09</td>
<td>6.12</td>
<td>--</td>
</tr>
</tbody>
</table>
### Table 11. Baseline solution and media nutrient concentrations collected from Environment 2 prior to seeding.

<table>
<thead>
<tr>
<th></th>
<th>Inorganic N</th>
<th>Urea</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Bicarbonate</th>
<th>Phosphorus</th>
<th>pH</th>
<th>Soluble Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media</strong></td>
<td>45.68</td>
<td>0.10</td>
<td>1.20</td>
<td>44.50</td>
<td>--</td>
<td>0.10</td>
<td>4.37</td>
<td>162.75</td>
</tr>
<tr>
<td><strong>Solution</strong></td>
<td>4.12</td>
<td>0.12</td>
<td>0.51</td>
<td>3.61</td>
<td>0.35</td>
<td>0.09</td>
<td>6.21</td>
<td>--</td>
</tr>
</tbody>
</table>
Figure 1. Bicarbonate concentration in the float bed solution in environment 1 and 2. Dotted line represents aerated treatment. Solid line denotes non-aerated treatment. Dashed line at 2 meq/L represents bicarbonate threshold, above which seedlings can show toxicity symptoms. Data are reported by individual environment.
Figure 2. Dissolved oxygen concentration in the float bed solution from Environment 1 and 2. Dotted line represents aerated float bed solution. Solid line denotes non-aerated float bed solution.
Figure 3. Nitrate Concentration in the float water solution from Environments 1 and 2. Dotted line represents aerated treatment. Solid line denotes non-aerated treatment. Data are reported by individual environment.
Figure 4. Ammonium concentration in the float water solution from Environments 1 and 2. Dotted line represents aerated treatment. Solid line denotes non-aerated treatment. Data are reported by individual environment.
Figure 5. Inorganic nitrogen concentration in the float water solution. Dotted line denotes aerated float water solution. Solid line denotes non-aerated float water solution. Data are reported by individual location.
Figure 6. pH of the float water solution in Environments 1 and 2. Aerated float water solutions are represented by a dotted line. Solid lines denote non-aerated float water solution. Data are reported by individual environment.
Figure 7. Urea concentration in the float water solution. Dashed line denotes aerated float water solution. Solid line denotes non-aerated float water solution. Data are reported by individual environment.