## ABSTRACT

DRAKE, MATTHEW PAUL. Evaluation of Nitrogen Application Timings on Yield, Quality, and Sucker Control. (Under the direction of committee chair Dr. Loren R. Fisher).

Accurate nitrogen applications are important for flue-cured tobacco production in the US. Two studies were conducted in NorthCarolina in 2012 and 2013 to evaluate the effects of nitrogen rate, nitrogen application timing, and nitrogen application method on the yield, quality, and leaf chemistry aspects of flue-cured tobacco. The purpose of this research was to evaluate current application rates, timings, two different nitrogen application methods likely to be used in a production setting, and the benefits that liquid nitrogen provide inthe flexibility of application .

The first study reviewed the effects of various nitrogen rates applied at differing time intervals through flowering of the plant. Yield, quality, and leaf chemistry data were analyzed. Treatments included rates above, at, and below recommended nitrogen levels for specific fieldenvironments. Treatments were applied at different intervals; timings included combinations of at transplanting, two weeks after transplanting, lay-by, two weeks after lay-by, and topping. In addition to yield, quality, and leaf chemistry data, tissue samples of green leaf tobacco were taken to evaluate nitrogen content and SPAD meter readings were taken to quantify greenness of the leaf.

Crop yield, quality, and value were not affected by treatments across all environments in the rate study under the environmental conditions of these studies. Nitrogenwas applied later in the season than current production practice without affecting agronomic aspects of the leaf.Nitrogen content of the leaf at layby, nitrogen content of the leaf at topping, total alkaloids, andSPAD readings measurements were all typically higher for treatments that had

either a late season nitrogen application or a higher rate of nitrogen applied.Nitrogen content, total alkaloids, and SPAD measurements increased with higher rates of nitrogen. Reducing sugars were greater with lower nitrogen rate treatments.

The second study evaluated the effects of application method. Two different application methods were tested.Late season applications of nitrogen were applied directly to the soil to simulate a drop-line application or were applied directly to the leaf foliage. The shape of a tobacco plant funnels liquids to the base of the plant, allowing the nitrogen solution to reach the rooting zone.Applications were made at a time to correspond with topping of the tobacco plant, approximately eight weeks after transplanting.

A yield increase was observed with soil applied nitrogen treatments. Leaf quality was affected by method of application and timing but no exact correlation method or timing could be concluded.Nitrogen application timing and nitrogen method affected the value per area. Total alkaloid levelswere not affected by treatments. Reducing sugar levels typically increased for treatments with earlier applied nitrogen. Leaf nitrogen content at layby was highest for treatment timings that had more than 50% of the required nitrogen applied by layby stage of growth. Leaf nitrogen content at topping and SPAD measurements both were for foliar applied nitrogen when compared to soil applied nitrogen.

Rainfall and temperature played a major role in the outcome of this research. Based on current knowledge and information received from this research late season nitrogen application is a useable tool but should be done with caution due to the possibility of decreasing leaf quality. If similar precipitation amounts are not observed, it has not been determined if using thathigh of a rate of nitrogen that late in the season would be recommended.

# Evaluation of Nitrogen Application Timings on Yield, Quality, and Sucker Control

by
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## DEDICATION

This thesis is dedicated to my Grandfather, Almond Jerkins Drake II, for his persistence in me going the extra mile and following through with any commitment. To my father Paul Drake, for encouraging me to expand my horizons, learn all that I can, and never settling for anything less than perfect. To my mother Melissa Drake, who supported me through every step in life, who never gave up on me even when there seemed to be no easy way out. To both of my loving sisters Katherine Stowe and Morgan Drake, I would not have made it out of Chemistry 101 or learned how to enjoy the little things in life without either of you.

## BIOGRAPHY

Matthew Paul Drake, son of Paul and Melissa Drake, was born in Pinetops, North Carolina on October 30, 1989. He worked on the family farm as a fourth generation participant and graduated from SouthWest Edgecombe High School in 2008. The family farm played an important role in him pursuing a Bachelor's of Science Degree in Agricultural Business Management from North Carolina State University in 2012 with a minor in Animal Science. After graduation he immediately started working towards his Master of Science Degree in the Crop Science Department at North Carolina State University. Under the direction and leadership of Dr. Loren Fisher, Dr. Randy Wells, and Dr. David Smith he expanded his interest in tobacco production with several research projects. Upon completion he plans to continue in the tobacco industry as an agronomist for Universal Leaf Tobacco Company

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# CHAPTER ONE

## INTRODUCTION

Nitrogen is an essential macronutrient that is required by plants in adequate amounts. Nitrogen is the building block of all proteins, of metabolic pathways that are involved in synthesis and energy transfer, and in the synthesis of nucleic acids. When soil moisture is adequate, nitrogen normally is the major limiting factor for crop production. (Haynes, 1986; Olson and Kurtz, 1982).Although there is an abundance of dinitrogen gas in the atmosphere, it is commonly deficient in soils in the oxidized (nitrate) and reduced (ammonium) forms which are available for plant absorption (Novoa and Loomis, 1981).Both vegetative and reproductive growth in plants will be reduced without adequate amounts in a plant useable nitrogen.

### Soil Nitrogen

Most plants that have chlorophyll as the dominant leaf pigment exhibit a pale green colorwhennitrogen deficient, andis the most common deficiency symptom observed in such plants. Plants with these symptoms will respond dramatically to applications of nitrogencontaining fertilizers (Troeh and Thompson, 2005). Application(s) of nitrogen in many cropping systemsis required as native soils will not supply the nitrogen that is needed by the plant.The ultimate source of nitrogen used by plants is $N_2$ gas, which makes up 78% of the earth's atmosphere (Havlin et al., 1999). Higher plants are incapable of metabolizing$N_2$ gas directly into metabolically useable fixed nitrogen. $N_2$ gas must be

transformed into plant available nitrogen by one of the four following steps; biological nitrogen fixation, mineralization, atmospheric electrical discharges forming nitrogen oxides, or the commercial production of asynthetic nitrogen fertilizerthrough the Haber-Bosch process (Havlin et al., 1999).

The majority of nitrogenin soil is partitioned in soil organic matter (Troeh and Thompson, 2005). The organicnitrogen in soils is unavailable to plants and only becomes availablewhen it is mineralized by microorganisms. Microbial activity will decompose the organic material into plant useable inorganic nitrogenions. However, the rate of nitrogen use by growing plants can surpass the rate at which nitrogen becomes available from this process (Troeh and Thompson, 2005).While most of the soil nitrogen comes from decayed plants and animals, this is not the primary source for plant growth. The primarynitrogen source is the atmosphere(Troeh and Thompson, 2005). Originally the soil nitrogenwas likely fixed by lightning and then entered the soil profile through rain water. This process is continuous, onlyadding approximately 5-10 pounds of nitrogen per acre (5-11 kg/ha) per year(Troeh and Thompson, 2005). The limited amount provided from the atmosphere through lighting is not enough nitrogen for proper plant growth (Stevenson, 1982). The ultimate source of fixed atmospheric nitrogen for plant nutrition is from the Haber-Bosch process which produces ammonia in the presence of an iron-based catalyst, high heat and high pressure (Modak, 2011). The ammonia formed can be used to produce various nitrogen-based fertilizers including nitrate based products through the oxidation of ammonia. Both ammonium and nitrate can then be absorbed by growing plants (Troeh and Thompson, 2005). Therefore, with

inorganic nitrogen requirements being much greater than what can be supplied by natural fixation of organic nitrogen to plants, growers must supply crops with additional nitrogen to complete their growing cycle from germination to harvest. Both ammonium and nitrate can be blended or complete fertilizers supply the plants with essential nutrients and other micronutrients that are required for proper plant growth (Troeh and Thompson, 2005). Commercially produced synthetic fertilizers are the most important sources of nitrogen for plant growth (Havlin, et al., 1999). Havlin et al. (1999) reported that "Over the last 20 years, world nitrogen consumption has increased from 22 to 80 million metric tons."

Cell formation requires nitrogen in growing plants. Carbohydrates from carbon dioxide and water will be produced by photosynthesis and with nitrogen and other essential nutrients the synthesis of proteins (including enzymes), nucleic acids, and lipids is possible and are the building blocks for growth (Troeh and Thompson, 2005). More importantly nitrogen must be available in a usable form at the correct plant growth stage. Plant available nitrogenis a must for optimal production, making inorganic nitrogen a necessity.

**Nitrogen Uptake**

Any ion that is absorbed by the plant root must come into contact with the root surface. Three major pathways facilitate ion absorption by the plant,including 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 lower concentration), and mass-flow (nutrient movement with soil water movement)(Tisdale,

1975).Nitrogen has numerous pathways that allow it to enter or leave the soil-plant system. There are more pathways for nitrogen movement in and out of the soil-plant system than any other nutrient (Stevenson, 1982). Unlike many other nutrients,nitrogen can be taken up as a cation as ammonium ($NH_4^+$) or an anion as nitrate($NO_3^-$). The rate of absorptionis normally dependent on the availability of the two ions in the solution. For generalpurposes one $NH_4^+$ may substitute for one $NO_3^-$ and *vice versa.*Uptake rates are determined by the physiological needs of the plant, not the charge or form of the ion (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 of nitrogen is regulated by both the chemical and spatial availability of nitrogen in the soil. It is also affected by the activity and number of transport systems, movement from source to sink, and utilization needs depending on the growth phase (vegetative or storage) of the plant (Engels and Marschner, 1995). There are different uptake pathways for both nitrate and ammonium. When the concentration of nitrate is higher in soil solution (outer concentration), then a passive transport along the electrochemical potential gradient via nitrate specific channels results. When the outer concentration is lower a carrier-mediated symport ($NO_3^-/H^+$) or an antiport ($NO_3^-/OH^-$) is used (Engels and Marschner, 1995). It has been suggested that with eithera high or low concentration, a facilitated transport system may be a more accurate description of the movement of nitrate (Engels and Marschner, 1995). This concept is supported from the depolarization of the transmembrane potential of epidermal and cortical cells in roots of barley plants, suggestingan active

transport regardless of the concentration (Engels and Marschner, 1995; Glass, et al., 1992). The uptake of nitrate which carries a negative charge is associated with a depolarization of the plasma membrane. The cytosol is neutralized from a net negative charge to a net neutral charge. Charge neutralization takes place asnitrate is transported into the cytosol. The neutralization develops as protons are transported with the nitrate as it moves into the cytosol causing a pH increase in the soil solution(Mengel and Kirkby, 2001;Ullrich and Novacky, 1981; Smiley, 1974).Ammonium is a cation therefore it does not require metabolic energy to be taken up by plants. It will follow the electrochemical gradient between the apoplasm and symplasm. Ammonium may enter the plant through membrane penetration by diffusion or through ion channels in the cell walls (Engels and Marschner, 1995).

There are differences between the uptake of nitrate and ammonium. Uptake of nitrate is more dependent on the energy status of plants and is transported at high rates to upper portions of the plant and stored in the vacuole for later use (Mengel and Kirkby, 2001).When the plant is going through higher rates of photosynthesis due to absorption of sunlight the plant synthesizes more carbohydrates or energy leading to a potential increase in the uptake of nitrates (Rufty, 1989). The two ions are both sensitive to pH.Nitrate absorption occurs at lower pH because of the availability of protons in soil solution. Ammonium ions are taken up more readily at neutral soil pH (Mengel and Kirkby, 2001). Plant uptake is also known to vary between species and within genotypes. Uptake may vary within a genotype depending on time of day, plant age, nutrient status, and growth stage (Engels and Marschner, 1995). Rate of uptake may be affected depending on the relative source of nitrogen present. The rate

of ammonium uptake is not considered to be affected by the rate of nitrate presence in solution, however the rate of nitrate uptake can be lower when there ammonium is present in the soil solution (Haynes, 1986; Huffaker and Rains, 1978; Jackson, 1978).

**Nitrogen Transport within the Plant**

Since there are two different forms of nitrogen that are absorbed by the plant, different transport systems for nitrate and ammonium exist. Nutrients that are to be used by the plant must move to and then through the root tissue before they enter the xylem to be transported throughout the plant (Haynes, 1986). Nitrogen is brought to the roots through diffusion and mass flow (Havlin et al., 1999) and is either assimilated in the roots or translocated in the xylem to the upper plant tissue. The nitrogen form transported is regulated by the nitrogen uptake form and root metabolism (Mengel and Kirkby, 2001). Ammonium that is absorbed by the roots is assimilated in the roots and therefore is not translocated as ammonium but in the form of amino acids to the tissue portions of the plant (Mengel and Kirkby, 2001). If nitrate is not stored in the roots or assimilated there, then it is transported through the xylem to leaf tissue cells where it is stored or assimilated through the nitrate/nitrite reductase enzymes (Barker and Bryson, 2007).

Nitrate and amino acids are the major forms of nitrogen that are transported throughout the plant vascular system (Mengel and Kirkby, 2001). The need for nitrogen assimilates may determine how nitrogen is transported in the plant (Engels and Marschner, 1995). If there is a high demand for nitrogen and nitrogen assimilates then nitrogen is sent to

the shoot portions of the plant through the xylem and very little is recycled back through the phloem signaling a need for increased uptake (Engels and Marschner, 1995). If the plant is in low demand then nitrogen and nitrogen assimilates are recycled in a greater quantity back to the roots signaling a reduction in nitrogen uptake and limited amounts may be transported out of the roots (Engels and Marschner, 1995). Nitrogen is a mobile nutrient, if nitrogen deficiency is signaled while the plant is actively growing nitrogen from older leaves will be mobilized and translocated to the younger portion of the plant (Baker and Bryson, 2007). Once nitrogen has been assimilated or stored it is available for amino acid production depending on the plant needs.

**Nitrogen Assimilation within the plant**

Whilenitrogen that is considered plant available is 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).

Nitrate may follow several pathways before it is assimilated. To start, nitrate absorption is regulated by the balance of the influx of nitrate from the apoplasm to the cytoplasm (symplasm) and the opposite efflux actionfrom the cytoplasm to the apoplasm.Once this balance has been reached nitrate entering into the symplasm can either efflux back into the apoplasm or follow a specific pathway before it is assimilated (Lea, 2001). Nitrate can be stored in vacuoles in the roots, translocated in the xylem and stored in vacuoles of leaf tissue, reduced by nitrate/nitrite reductaseand stored in the roots, or

translocated through the xylem and then reduced by nitrate/nitrite reductaseand stored in the leaf tissue (Engels and Marschner, 1995; Barker and Bryson, 2007). Before it can be used, nitrate must be reduced in a two-step process that involves two enzyme-catalyzed reactions that occur in either the roots or the leaf tissue (Havlin et al., 1999). First, the nitrate reductase enzyme complex catalyzes the reduction of nitrate to nitrite with the addition of NADH or NADPH which are reduced pyridine nucleotides and an additional hydrogen ion. This process forms nitrite and $NAD^+$ or $NADP^+$ which are oxidized pyridine nucleotides (Barker and Bryson, 2007; Haynes, 1986). It should be noted that molybdenum is required as a cofactor and that the NADH or NADPH is a source from respiration in the roots along with photosynthesis in the leaves depending on where the reduction takes place (Barker and Bryson, 2007). Next, nitrite is then further reduced to ammonium with the ferredoxin-nitrite reductase enzyme. Ferredoxin-nitrite reductase is also the catalysis for the reduction of hydroxylamine to ammonium (Barker and Bryson, 2007; Haynes, 1986). The nitrite reduction takes place in one transfer in either the roots or leaves of the plants. The needed reducing potential comes from glycolysis or respiration if the process is taking place in the roots. If taking place in the leaf tissue the reducing potential comes from the chloroplast if nitrite is being reduced (Baker and Bryson, 2007). Onceammonium is produced it must be further assimilated before it can become useable by the plant.

It makes little difference if the ammonium comes from uptake from soil solution or if it comes from the nitrite reduction pathway, it still undergoes assimilation (Engels and Marschner, 1995). Ammonium assimilation takes place in the mitochondria of the roots if the

ion has been absorbed from the soil solution, if the ammonium is from nitrite reduction in the leaves it is assimilated in the chloroplasts portion of the cell (Baker and Bryson, 2007).Glutamic acid formation is a major entry way of inorganic nitrogen to organic nitrogenous compounds. There are two enzymes that make this processes possible; glutamine synthetase and glutamate synthetase. Ammonium, glutamate, ATP and reduced ferredoxin form glutamine and oxidized ferredoxin in the reaction catalyzed by glutamine synthetase. Glutamine and α-oxoglutarate form two glutamates in the glutamate synthase process. Both of these enzymes assimilate most of the ammonium that comes from absorption, reduction of nitrate, dinitrogen fixation, or photorespiration even if ammonium is at low concentrations because of the high affinity between glutamine synthetase and ammonium (Baker and Bryson,2007). If ammonium is at a high concentration a different pathway referred to as glutamic acid dehydrogenase is followed and glutamate and oxidized pyridine nucleotides are formed (Baker and Bryson, 2007). The assimilation processes of nitrate and ammonium allows plants to transport and use nitrogen as the building blocks for other life functions.

**Functions of Nitrogen in the Plant**

Proteins are important for numerous functions in the plant. They include making up the cell structure and for the directing and controlling of chemical reactions that make up the processes of a living plant (Lea, 2001). Amino acids are the building blocks of proteins, with proteins being composed of several copies of 20 possible amino acids linked in long chains (Lea, 2001). Nitrogen is a major component in an amino acid as it comprises the portion of

the amino acid that is considered the "basic amino group" ($-NH_2$). In combination with an acidic carboxyl group (-COOH), the two groups combined form an amino acid (Lea, 2001). As described earlier, the process of nitrate/ammonium assimilation is how plant available inorganic nitrogen becomes useable by the plant through the glutamine/glutamate synthase processes. Once assimilated glutamate functions as the amino donor for several transamination reactions (Mengel and Kirkby, 2001). Most of the nitrogen that is present in green plants originates from the amino nitrogen of the glutamate produced in the glutamine plus 2-oxoglutarate reaction (Mengel and Kirkby, 2001).Amino acids can be synthesized through the transamination processes where glutamate and an oxyacid form an oxoglutarate and an alpha-amino acid (Barker and Bryson, 2007). Glutamate combined with ammonium and ATP form glutamine (an amide) and ADP. Amides are the nitrogen rich transportation or storage compounds that aid in accumulating excess nitrogen as it is recycled within the plant system (Barker and Bryson, 2007). Proteins are formed in the following sequence from the unique sequence of amino acids. Messenger ribonucleic acid (mRNA) attatch to Ribosomes that has entered into the cytoplasm. The ribosomes then allow two molecules of aminoacyl-tRNA to bind to the mRNA. The two amino acid residues are linked by the formation of peptide bonding as the ribosome moves down the mRNA. It releases the first tRNA and allows the next aminoacyl-tRNA to bind to the sequential position along the mRNA forming an elongated peptide/protein (Marschner, 1995). Protein formation is only one function of amino acids. Individual amino acids have specific functions within the plant. Arginine is a major nitrogenstorage compound within plants and proline has been suggested to function as

an osmoticum in plants. Both arginine and proline are derived from glutamate (Lea, 2001). The aspartate pathway which forms lysine, isoleucine, threonine, and methionine is of essential importance as these four amino acids are an essential part of the nutritive base of plant seeds (Lea, 2001). The shikimate pathway which synthesis tryptophan, phenylalanine, and tyrosine is relevant in that this pathway is the precursor for the formation of secondary metabolites such as phenolic acids, flavonoids, and alkaloids (Lea, 2001).In combination, the organically bound nitrogen of glutamate and glutamine is used for synthesizing other amides, ureides, amino acids, amines, peptides, and proteins (Marschner, 1995). Many vital functions are dependent on nitrogen within the plant. Proper nitrogen nutrition is of upmost importance for proper plant growth and maturity (Mengel and Kirkby, 2001).

**Function and Role of Nitrogen in Flue-cured Tobacco**

Nitrogen can be considered the most important macronutrient in flue-cured tobacco (*Nicotianatabacum* L.) production.Appropriate levels are needed to produce a high yielding, quality leaf, with appropriate aroma and smoke flavor (McCants and Woltz, 1967). Flue-cured tobacco will accumulate approximately fifty percent of the total nitrogen absorbed in a season within five weeks of transplanting with the majority of the nitrogen being absorbed between the fourth and fifth week. The remainder will be accumulated within eight to nine weeks after transplanting (Raper and McCants, 1966).Nitrogen must be applied in adequate amounts and be available to the plant during the essential stages of growth to allow the greatest influences on chemical properties of the leaf (Chaplin and Miner, 1980).Ripeness of

the leaf is greatly influenced by nitrogen availability during the growing season. Excess nitrogen will delay ripeness and cause the leaf to become “dark brown to black in color and dry and chaffy” (McCantz and Woltz, 1967). Deficiencies of nitrogen can cause immature tobacco leaves to appear ripe if only the color of the leaf is being evaluated. However, the cured leaves of nitrogen deficient tobacco will be pale in color and lack both physical and chemical properties that are common in quality tobacco leaf (McCantz and Woltz, 1967). Maturity of the tobacco plant is correlated with the ageof the plant while ripeness is a factor of nitrogenattenuation(Fisher, 2013). Nitrogen depleted leaves can appear ripe but if they are physically immature they will not cure properly. Also, an unripe leaf may be mature but due to excess nitrogen may not be considered ripe therefore, if harvested will not produce a quality leaf. Due to the unique harvesting of the vegetative portion of the tobacco plant, starting from the base moving towards the top portion of the plant, leaves at different stalk positions will be at different physiological growth stages at any specific point in time (McCantz and Woltz, 1967).It is important that as the plant reaches its maximum leaf area thenitrogen in the system starts to decline for proper leaf quality (McCantz and Woltz, 1967). This decline in nitrogenconcentration after maximum leaf area is reached allows for the harvest of properly ripened leaves without losing yield potential.

The correct proportion of reducing sugars to total alkaloids is important for the taste and aroma of tobacco (Tso, 1990b). Nicotine is one of the major alkaloids that is desirable in tobacco (Collins and Hawks, 1993). Therefore, the proper rate of nitrogen is important to produce reducing sugars and nicotine ratio that is about 6 to 8:1 (Flower, 1999). Under

fertilized tobacco or tobacco that has been subject to leaching have higher ratios of reducing sugars to total alkaloids. Over fertilized tobacco or tobacco that has been subject to drought have lower ratios of reducing sugars to total alkaloids (Flower, 1999). Nicotine and other chemical constituents to smoke characteristics are produced through the sikimate pathway as discussed earlier (Lea, 2001).

Different types of tobacco are grown on different soils depending on nutrient requirements of the tobacco type. Flue-cured tobacco is generally grown on sandy loams and sandy soils (Flower, 1999). With this variation in soil types,nitrogen recommendations vary depending on the soil type. Tobacco grown in sandier soils may require80 pounds of nitrogen per acre while soils with a greater percentage of clay may only require 50 pounds of nitrogen per acre (Collins and Hawks, 1993). Current recommendations still follow these rates to achieve optimum quality and yield (Vann and Smith, 2014). Nitrogen is the second most absorbed nutrient next to potassium in flue-cured tobacco but is the key nutrient in tobacco fertilization (Flower, 1999). Since the soils where flue-cured tobacco is grown are typically sandy and well drained, organic matter is typically low and therefore soil nitrogenreserves are usually low, adding to the importance ofproper nitrogen application rates (Peedin, 1999). Soil amendments are controlled by the grower but weather conditions are not. Nitrogen is leachable in well watered soils and tobacco grown in heavily leached soils is associated to high sugar, low nicotine tobacco (Peedin, 1999).Drought stressed tobacco is just the opposite with low sugar and high nicotine content (Peedin, 1999).Neither of which are desirable in a quality crop. Due to the leaching potential of nitrate many growers have changed over the

past decade what was considered common production practices. Banding of nitrogen is now common in comparison to a broadcast application of fertilizer (Tso, 1990a).The use of urea ammonium nitrate (UAN) as a portion/entire amount of the required nitrogen has also become a popular practice (Vann and Smith, 2014). These practices reduce the chances of leaching which aid in the production of quality leaf. Using an ammonium based fertilizer aids in the reduction of leaching potential as it carries a positive charge which binds to the negatively charged soil colloids. Leaching is reduced until the ammonium in transformed into nitrate form of nitrogen. This transformation is mainly dependent on soil moisture and soil temperature.Liquid sources of nitrogen are easier to handle and are generally cheaper than dry sources. Transitioning from a granular blended fertilizer to a UAN product can save the grower potentially 40-45% on his/her fertilizer cost (Vann and Smith, 2014).

The form of nitrogen needed in tobacco production has long been debated. Early work done by McCants and Woltz(1963 and 1967) and many others indicated that when nitrate is less than 30 percent of the total nitrogen source applied then reduced growth can occur (Peedin, 1999).More recent research indicates there are no significant differences in neither agronomic northe leaf chemical characteristics when the nitrogen source is 100 percent UAN. Parker (2009) verified that nitrogen source did not affect yield, grade index, value, total alkaloids, total reducing sugars, or leaf color (Parker, 2009). Switching granular fertilizers to either all or a portion of the nitrogen applied fromUANcan help the grower reduce nitrogen loss in a cropping system and decrease the input cost for the crop without compromising yield or quality.

**Justification of Research**

The first crop of tobacco in the US was grown in 1612 by the Jamestown colonist (Collins and Hawks, 1993). Ever since the production and distribution of the unique leaf, tobacco has been a mainstay for thousands of families throughout the past few centuries (Collins and Hawks, 1993). Flue-cured tobacco originated somewhere between 1826-1839 depending on which historian is explaining the details of how flue-cured tobacco came to be produced (Collins and Hawks, 1993). US flue-cured tobacco production since 2009 has fluctuatedbetween 156 to 239 million kilograms of cured leaf grown on approximately 80,000 hectares each year (Brown and Snell, 2014).

As discussed throughout the chapter nitrogen is essential for proper plant growth and an absolute must in tobacco production. With research previously conducted on fertility and nitrogensource the next step for current production was to determine if it was possible to split nitrogen applications into multiple applications. This is altering the common “split application” method by adding nitrogen in a “spoon feeding manner.”The majority of growers will apply the first fertilizer application as either a band or a broadcast granular or liquid fertilizer before transplanting or just after transplanting. If the fertilizer is broadcast it is done pre-transplant and then cultivated into the soil profile. If it is banded, it is usually applied within one week of transplanting. A furrow is opened and the fertilizer is either dropped or sprayed into the furrow and then incorporated into the soil profile. In the banded operation, fertilizer is placed approximately 12 cm from the roots of the transplants to reduce possibility of fertilizer salts injury (Collins and Hawkes, 1993). The second application is

done just before or at the lay-by growth stage of the plant (three to four weeks after transplanting). The remainder of the needed nitrogen (and possibly other nutrients) is added in the same banded method described above.

Applying nitrogen in more than two applications would allow growers to base nitrogen rates on current growing conditions in addition to previous field experience. Currentnitrogen rates applied to the tobacco crop are based on soil type, cultural practices, and previous field history (Vann and Smith, 2014). When a grower has a season that has less than average rainfall, there is typically too much nitrogen in the soil profile and can lead to less mature leaves that have had a delay in ripening. When there is excess rainfall, immature leaves may appear to be ripe even if they are not from a physiological growth standpoint thus reducing yield and quality.With nitrogen availability being so closely correlated to weather conditions, and tobacco leaf quality being directly related to nitrogen availability, a new nitrogen regiment of more than two applications in a given year could assist the grower in producing quality tobacco without reducing yield. Many producers determinenitrogen needs on experiences from previous years. Multiple split applications (up to as many as 4) could help the grower base the nitrogen needs of the crop on the current weather and field conditions not average needs in previous crop years. As stated earlier tobacco nitrogen needs/uptake are greatest (about 75 percent total nitrogen absorbed) between four and nine weeks after transplanting (Raper and McCants, 1966). Banding a smaller portion of the total nitrogen needed earlier and then coming back multiple times just before the four week stage for another portion of the nitrogen and then again before the nine week stage may prove to be

an efficient method in continuously supplying nitrogen to the plant without losing nitrogen to leaching. The reverse is also possible where if the grower has had less rain in a given year then the final application may not be warranted. This method would allow growers to better regulate nitrogen supplies to the plant and possibly save in the cost of fertilizer if less fertilizer is applied. In addition to determining if multiple applications are beneficial, application method of the late season nitrogen is also important. It needs to be determined if application method affects yield, quality, and value of the crop. Both soil applied nitrogen and foliar applied nitrogen need to be considered.

Infrastructure in the tobacco production regions is most likely at a maximum with little ability to expand acreage due to expansion cost, competitive commodity prices, and other barriers of entry into tobacco production (Fisher, 2013). Due to this lack of infrastructure, extended growing seasons, and large acreage growers having problems handing the volume of the crop in the later part of the season when much of the leaf is removed. A late season application of nitrogen could be used to delay ripening and add holding ability in the delaying of ripening of the plant. This delay in when the leaf is ripening could help keep the leaf on the plant longer without losing leaf mass. Barn space and barn utilization could be better controlled if this method allowed portions of the crop to mature at different timing intervals. As barn space is thought to be needed the grower could apply a late season nitrogen application to a portion of the crop and possibly delay ripening. This would allow barns to have time to become available before the leaf starts to loose leaf mass

and have a decrease in quality. This late season application can also be vital in understanding when is too late to apply nitrogen.

The possibility of a late season application is practical using high clearance sprayers. Some growers historically have used fertilizer spreaders to apply a granular fertilizer over the top of the crop. With fewer farms leaving vacant rows in the field this is not as easy as before as well as the possibility of granules getting caught in the leaf axil.Damage caused by fertilizer in the leaf axil can to lead to fragile leaves, resulting in leaf drop. High clearance sprayers can either apply liquid nitrogen to the soil surface using drop-lines or liquid nitrogen can be applied directly to the leaf foliage similar to how plant growth regulators used for control of axillary bud growth in tobacco are applied.Plant growth regulators are applied at a high volume of water per area (approximately 465 liters per hectare) with low pressure. This is to ensure proper distribution of contact type regulators that must reach each of the plants axillary buds. The method of using high clearance sprayers is a more efficient way to apply fertilizer when compared to using a spreader for granular type fertilizers. The grower can cover more area per trip and handling of liquid products is generally easier. Also the dilution of the nitrogen in the water has less of a chance to affect the leaf axil resulting in less of a chance for leaf drop. Assuming the concentration is not too high,necrosis of the leaf tissue is not expected. There is currently no information on the efficiency of either of these methods therefore determining if there are any differences is necessary to validate either or both methods so extension personnel can make proper recommendations to the grower.

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# CHAPTER TWO

# NITROGEN APPLICATION RATE AND TIMING EVALUATION ON YIELD, QUALITY, AND CHEMICAL COMPONENTS OF FLUE-CURED TOBACCO (*Nicotianatabacum*L.)

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**ABSTRACT**

Research was conducted at two locations in 2012 and three locations in 2013 to determine the effect of nitrogenapplication rate and timing on yield, quality, and leaf chemistry of flue-cured tobacco. Treatments included rates above, at, and below recommended nitrogen levels for specific field locations and were applied at differing intervals starting at transplant and continuing until topping.Urea ammonium nitrate was the nitrogen source that was used across all treatments. Yield data were taken and quality of the leaf was determined by a USDA grade. Crop value per hectare was determined using the quality grade, based on market price for specific grades.

Tissue samples were taken at layby (when plants are roughly 38 cm high), at topping (approximately 60-70 days after transplant), and after curing to evaluate total nitrogen content of the leaf at the specified growth stages. Tissue samples of the cured leaf were analyzed for both total alkaloids and reducing sugars. All data were subjected to analysis of variance (ANOVA) using PROC GLM procedure in SAS. Treatment means were separated using Fisher's Protected LSD test at $p \leq 0.05$.

Crop yield and quality were not affected by treatments across all environments. Due to the lack of effect on crop yield and quality, value per hectare was also not affected under the environmental conditions of this study.Nitrogen rates are adequate depending on location of production. Nitrogen can be applied later in the season than current production practice without affecting agronomic aspects of the leaf if similar weather events and rainfall amounts occur. Nitrogen content of the leaf at lay-by and topping, total alkaloids, reducing sugars, and

SPAD readings were all affected by the application timing and rates. Typically as rate of nitrogen increased alkaloid levels increased and reducing sugars decreased and *vice versa.* Nitrogen leaf content at topping and SPAD measurements were highest in plots that received a nitrogen application later in the season.

Rainfall and temperature played a major role in the outcome of this research. Based on current knowledge and information received from this research late season nitrogen application is a useable tool but should be done with caution due to the possibility of affecting leaf quality.

## NOMENCLATURE

*Nicotianatabacum*L., tobacco, urea ammonium nitrate.


## KEY WORDS

Nitrogen, nitrogen rate, nitrogen timing, UAN.


## INTRODUCTION

### Function and Role of Nitrogen in Flue-cured Tobacco

Nitrogen can be considered the most important macronutrient in flue-cured tobacco (*Nicotianatabacum* L.) production. Appropriate levels are needed to produce a high yielding, quality leaf, with appropriate aroma and smoke flavor (McCants and Woltz, 1967). Flue-cured tobacco will accumulate approximately fifty percent of the total nitrogen absorbed in a season within five weeks of transplanting with the majority of the nitrogen being absorbed between the fourth and fifth week. The remainder will be accumulated within eight to nine weeks after transplanting (Raper and McCants, 1966). Nitrogen must be applied in adequate amounts and be available to the plant during the essential stages of growth to have the greatest influence on chemical properties of the leaf (Chaplin and Miner, 1980). Ripeness of the leaf is influenced by nitrogen in the season and ripeness is correlated with leaf qulaity. Excess nitrogen will delay ripeness and cause the leaf to become “dark brown to black in color and dry and chaffy” (McCantz and Woltz, 1967). Deficiencies of nitrogen can cause immature tobacco leaves to appear ripe if only the color of the leaf is being evaluated.

However, the cured leaves of nitrogen deficient tobacco will be pale in color and lack both physical and chemical properties that are common in quality tobacco leaf and will not cure properly (McCantz and Woltz, 1967). Maturity of the tobacco plant is correlated with age of the plant while ripeness is a factor of nitrogen attenuation as the growing season progresses (Fisher, 2013). Also, an unripe leaf may be mature, but due to excess nitrogen may not be considered ripe therefore, if harvested will not produce a quality leaf. Due to the unique harvesting of the vegetative portion of the tobacco plant, starting from the base moving towards the top portion of the plant, leaves at different stalk positions will be at different physiological growth stages at any specific point in time (McCantz and Woltz, 1967). It is important that as the plant reaches its maximum leaf area that the nitrogen in the system starts to decline for proper leaf quality (McCantz and Woltz, 1967). This decline in nitrogen after maximum leaf area is reached allows for the harvest of properly ripened leaves without losing yield potential.

The correct proportion of reducing sugars to total alkaloids is important for the taste and aroma of tobacco (Tso, 1990b). Nicotine is one of the major alkaloids that is desirable in tobacco (Collins and Hawks, 1993). Therefore, the proper rate of nitrogen is important to produce reducing sugars and nicotine ratio that is about 6 to 8:1 (Flower, 1999). Under fertilized tobacco or tobacco that has been subject to leaching have higher ratios of reducing sugars to total alkaloids. Over fertilized tobacco or tobacco that has been subject to drought have lower ratios of reducing sugars to total alkaloids (Flower, 1999). Nicotine and other

chemical constituents to smoke characteristics are produced through the sikimate pathway as discussed earlier in the chapter (Lea, 2001).

Flue-cured tobacco is generally grown on sandy and sandy loam soils (Flower, 1999). With this variation in soil types, nitrogen recommendations vary depending on the soil type. Sandier soils may require 80 pounds of nitrogen per acre while soils with a greater percentage of clay may only require 50 pounds of nitrogen per acre (Collins and Hawks, 1993). Current recommendations still follow these same rates to achieve optimum quality and yield (Vann and Smith, 2014). Nitrogen is the second most absorbed nutrient next to potassium in flue-cured tobacco but is the key nutrient in tobacco fertilization (Flower, 1999). Since the soils where flue-cured tobacco is grown are typically sandier and well drained, organic matter is typically low therefore soil nitrogen reserves are usually less, adding to the importance of proper nitrogen application rates (Peedin, 1999). Tobacco that has received excessive rainfall will experience soils with heavily leached nitrogen and can result in high sugar, low nicotine tobacco (Peedin, 1999). Drought stressed tobacco is just the opposite with low sugar and high nicotine content (Peedin, 1999). Neither of which are desirable in a quality crop. Due to the leaching potential of nitrate many growers have changed what was considered common production practices in the past decade. Banding of nitrogen is now common in comparison to a broadcast application of fertilizer (Tso, 1990a). The use of urea ammonium nitrate (UAN) as a portion/entire amount of the required nitrogen has also become a popular practice (Vann and Smith, 2014). These practices reduce the chances of leaching which aid in the production of quality leaf. Transitioning from a granular

blended fertilizer to a UAN product can save the grower potentially 40-45% on his/her fertilizer cost (Vann and Smith, 2014).

The form of nitrogen needed in tobacco production has long been debated. Early work done by McCants and Woltz (1963 and 1967) and many others indicated that when nitrate is less than 30 percent of the total nitrogen source applied then reduced growth can occur (Peedin, 1999). Parker (2009) verified that nitrogen source did not affect yield, grade index, value, total alkaloids, total reducing sugars, or leaf color (Parker, 2009). Switching to either all or a portion of the nitrogen applied from UAN can help the grower reduce nitrogen loss in a cropping system and decrease the input cost for the crop without compromising yield or quality.

Current nitrogen rates applied to the tobacco crop are based on soil type, cultural practices, and previous field history (Vann and Smith, 2014). With research previously conducted on fertility and nitrogen source the next step for current production was to determine if it was possible to split nitrogen applications into multiple applications.Applying nitrogen in more than two applications would allow growers to base nitrogen rates on current growing conditions in conjunction with field history. With nitrogen availability being so closely correlated to weather conditions, and tobacco leaf quality being directly related to nitrogen availability, a new nitrogen regiment of more than two applications in a given year could assist the grower in producing quality tobacco without reducing yield. The present research was conducted to determine if splitting nitrogen applications into more than two

applications was beneficial for proper plant growth and to determine when the late season threshold for application occurs.

## METHODS AND MATERIALS

### Field Procedures

Research was conducted at the Border Belt Tobacco Research Station (BBTRS) near Whiteville, NC and the Oxford Tobacco Research Station (OTRS) in Oxford, NCin 2012. An additional location at the Upper Coastal Plains Research Station (UCPRS) near Rocky Mount NC in 2013.Given that each location within a year and locations from year to year behaveindependently of the other locations, a specific year plus the corresponding location will be referred to as an environment.Tobacco was produced using suggested practices according to North Carolina Cooperative Extension Service, based on specific growing environmentswith the exception of experimental treatments (Fisher, 2014). After curing, leaves were weighed to determine yield and assigned an USDA government grade. Government grades were based on leaf maturity and ripeness. The grades are assigned a value that is associated with a grade index value (Bowman et al., 1988).

Treatments consisted of nitrogen applications made in different combinations of timing and rate. Timings of nitrogen applications were based on growth stages of the plant. The lay-by treatment (approximately 38 cm height) and at topping (approximately 120 cm height) treatments were approximately four and eight weeks after transplant, respectively. The control for the study consisted of 50% of the recommended nitrogen applied at

transplanting and 50% of the recommended nitrogen applied at layby. The control was to simulate what the majority of tobacco growers are currently using. All treatments had 28% UAN applied to the soil surface using either a TG 5 or TG 3 full cone nozzle depending on the rate of nitrogen that was required. Treatments were applied using $CO_2$ backpack sprayer. Pressure ranged from 100-175 kPa depending on rate and nozzle size. Treatments were replicated four times and arranged in a randomized complete block design. There were four rows per plot that were 12.2 meters long and 4.9 m wide. Plant spacing between individual plants within the rows was 0.55 m in all environments. Tissue sampling and leaf harvestwere performed on the two center rows. The outside two rows served as border rows between treatments. Due to extreme rainfall in the 2013, season we did not evaluate one replication at both the Boarder Belt Tobacco Research Station and the Upper Coastal Plains Research Station.

Cultivar selection was dependent upon the specific environment and are listed in Table 1.. Tissue samples were taken at three time intervals, at layby, at topping, and from the cured leaf. Layby is approximately four weeks after transplanting and 38 cm tall and topping is approximately eight weeks after transplanting and 120 cm tall. Total nitrogen content of the leaf was assessed from all three timing intervals. The green leaf tissue (layby and at topping) samples were taken from either the third or fourth leaf from the apex of the plant. The leaves measured approximately 10 cm wide and 15 cm long and were taken from approximately five plants within each plot.After sampling, the tissue was dried, ground, and sent to the North Carolina State University Tobacco Analytical Services Lab for total leaf

nitrogen content analysis. Both layby and at-topping samples were taken approximately one week after the corresponding layby or at topping nitrogen treatments were applied. Chlorophyll was estimated using a Konica Minolta SPAD-502 meter and taken from this same leaf position on the same day that leaf samples were taken for the "at-topping" green leaf tissue samples. In each plot ten leaves were measured using a SPAD meter and then averaged across the entire plot. After curing, a composite sample across stalk positions of cured leaf was taken to be analyzed for nitrogen, total alkaloids, and reducing sugars.

**Field Conditions**

Field conditions (Culitvar, transplant date, pH, base nitrogen rates, and $K_2O$ rate) (Table 1) varied by environment as well as monthly rainfall (Table 2). Urea ammonium nitrate (28%) supplied all nitrogen for all treatments.Either a banded or broadcast application of K-Mag (0-0-22) for the potassium source was applied. Amount applied was based on soil test results for potassium. No additional phosphorus was added as all soil reports showed high to very high P-index values therefore no additional P is recommended (Vann and Smith, 2014). The K-Mag also supplied the magnesium (10.8%) and sulfur (22%) needed for proper plant growth (Fisher, 2014).

Nitrogen was applied to each plot depending on treatment and there were a total of ten treatments including the control (Table 3). Nitrogen application started at transplanting and was completed prior to or at topping depending on the treatment.. Possible application timings evaluated were at planting, at  15 cm plant height, at 38 cm plant height (layby), at

60 cm plant height and at 120 cm plant height (topping). Timings corresponded with approximately 0, 2, 4, 6, and 8 weeks after transplanting. Rates of nitrogen applied were determined by the individual environments. Within each environment, rates of nitrogen applied were 75% of the total recommended nitrogen rate (treatments 2, 4, 6, 8), 100% of the recommended nitrogen rate (treatments 1, 3, 5, 7, 10), or 125% of the recommended rate (treatment 9), (Table 3). The research stations established nitrogen rate was used to determine the required amount for each treatment.Nitrogen was soil applied using a simulated soil directed application technique and was then incorporated with a Danish tine rolling cultivator. The "at topping" application was done to simulate drop nozzles used by high clearance sprayers to apply nitrogen to the soil surface.

**Analytical Procedures**

*Nitrogen.*Total leaf nitrogen content was analyzed using the procedure ofNelson and Sommers1973) in 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 the sample size was ground to< 80-mesh material to improve nitrogen recovery and shorten measurement time for each sample (Nelson and Sommers, 1973).

*Total Alkaloidsand Reducing Sugars.*Total alkaloids and Reducing sugars were analyzed using Perkin-Elmer Autosystem XL Gas Chromatograph procedure in the North Carolina State University Tobacco Analytical Services Laboratory. This procedure

determines total alkaloids based on chromatographic peak response at the retention times of alkaloid standards (Lewis et al., 2012).

*SPAD-502 Measurements.* SPAD-502 measurements were taken using Minolta chlorophyll meter. SPAD-502 measurements are relative chlorophyll measurements that nondestructively measure red region transmittance peak of chlorophyll a and b (around 660 nm) of the leaf and calculate a numerical value (SPAD value). This value is proportional to the amount of chlorophyll present in the leaf (Technical Bulletin, 1989). Greener leaves can then be correlated to higher leaf nitrogen content (Yoderand Pettigrew-Crosby, 1995).

*Statistical Analysis.* Data for crop yield, quality, value, percent nitrogen at layby, percent nitrogen at topping, percent nitrogen of cured leaf, percent total alkaloids, percent reducing sugars, and SPAD measurements were analyzed in SAS version 9.3. All parameters listed were subjected to an analysis of variance (ANOVA) using the PROC GLM procedure and treatment means were separated in accordance with Fisher's Protected LSD test with a p-value set at $p \leq 0.05$.

## RESULTS AND DISSCUSION

There was no significant treatment by environment interaction for yield, quality, value, layby leaf nitrogen content, and total alkaloid percentage therefore, these variables were combined over environments (Table 4). Reducing sugar percentage, at topping leaf nitrogen content, and SPAD measurements all had a treatment by environment interaction so each environment was evaluated independently (Table 4).

**Yield, Quality, and Crop Value**

*Yield.*Cured leaf yield was not affected by rate or timing of nitrogen application for any of the treatments (Table 4, 5). Due to no differences being observed, current nitrogen recommendations are adequate for tobacco production in the diverse growing regions of NC (Vann and Smith, 2014). Excess rainfall late in the season in 2012 may have aided in the leaching of nitrogen from the soil profile. The leaching in 2012 likely contributed to a reduction innitrogen availability and a timely ripening of the leaf. Above average rainfall in 2013 may have leached much of the required nitrogen from the rooting zone but due to late season nitrogen applications the plant had the needed nitrogen available for proper growth and was able to yield similar to a year where less precipitation was measured. Both years excess precipitation,though it occurred at alternate portions of the growing season,likely contributed to the similar yield responses that were observed. As noted earlier we did remove a replication at both the BBTRS and the UCPRS in 2013 due to the complete loss of the plants in those specific areas of the fields.

*Grade Index.*Cured leaf quality which is determined from an USDA assigned grade index was not affected by rate or timing of nitrogen application (Table 4, 5). It has been noted that increasing nitrogen rates above the recommended nitrogen amount can lead to decreased quality and a delay in ripening (Felipe and Long, 1988). However, this was not observed in this study. It is plausible that the excess rainfall in both years leached nitrogen from the soil profile allowing normal ripening. In 2012, with later season rainfall any excess nitrogen in the profile would have leached from the effective rooting zone. In 2013, there

was most likely little to no nitrogen in the profile after early season precipitation due to leaching, therefore adding a late season nitrogen application did not add excess nitrogen to the soil profile. Later season applications in 2013 would be more accurately described as leaching adjustment though this was not the primary intention of the study.

*Crop Value.* As expected, given the lack of response for both yield and quality crop, crop value per hectare was not affected by the rate or timing of nitrogen application (Table 4, 5). Due the suspected impact of weather on yield and quality as described above it is probable that value would follow the trend of both of these variables. As yield or quality increase value per area will increase as well. Had excess rainfall not occurred both years, a value difference across treatments would be possible.

**Chemical Characteristics**

*Total Alkaloids.*A significant treatment response was observed when comparing treatments for total alkaloid percentages of the cured leaf (Table 4, 6). As nitrogen rate increases total alkaloids typically will increase (Bush, 1999). The highest total alkaloid percentages were from treatments that had 100% or 125% of the recommended rate of nitrogen. The four highest total alkaloid percentages also had a late season nitrogen application at the “at topping” timing interval.All treatment averages for total alkaloids fell within the percentage range of 1,500 tobacco cultivars that were evaluated at a single location grown under similar cultural practices (Chaplin, 1980).

*Reducing Sugars.* A significant treatment by environment interaction was observed for the reducing sugar percentage (Table 4, 7). Each environment was evaluated separately. A significant treatment effect was observed at the Oxford Tobacco Research Station in 2012. All other environments did not have a significant treatment effect. The first or second highest reducing sugar percentages at each environment was always one of the four treatments that represented 75% of the total recommended nitrogen rate. With an exception of the Oxford Tobacco Research Station environment in 2013 and the Upper Coastal Plains Research Station in 2013 the first and second highest reducing sugar percentages were from one of the four treatments representing 75% of the total recommended nitrogen rate. This inverse relationship of higher reducing sugar percentages in the leaf of tobacco plants with less nitrogen applied was reported by (Tso, 1990c). Nitrogen percentage is a direct indication of nicotine percentage. Therefore in a normal environment as nitrogen is decreased, alkaloid percentages (nicotine) should decrease and reducing sugar percentageswill increase (Tso, 1990c).Once again all treatment averages for total reducing sugar fell within the percentage range of 1,500 tobacco cultivars that were evaluated (Chaplin, 1980).

*Leaf Nitrogen Content at Layby.* A response was observed when comparing treatments for the leaf nitrogen content at layby growth stage (Table 4, 6). Differences observed are likely because not all of the treatments had all of the nitrogen applied to those plots at the time of sampling. There were treatments that had 50% of the nitrogen applied after the layby stage, these treatments had less leaf nitrogen content than treatments that had already had 100% of the recommended rate applied when the samples were taken.

*Leaf Nitrogen Content at Topping.* A significant treatment by environment interaction was observed for the leaf nitrogen content at topping (Table 4, 8).The highest nitrogen percentage at topping at all environments came from a treatment that had an "at topping" nitrogen application except for the Upper Coastal Plains Research Station in 2013. At the Upper Coastal Plains Research Station in 2013 the second highest nitrogen percentage occurred in a treatment that had an "at topping" nitrogen application. Within each environment, percent leaf nitrogen content varied only slightly.

*SPAD Measurements.* A significant treatment by environment interaction was observed for the SPAD measurements (Table 4, 9). There was a large amount of variability in the SPAD measurements from environment to environment. No clear relationships existed between treatments. With SPAD readings being indicative of chlorophyll, higher measurements were seen in most of the treatments that had higher rates of nitrogen applied.

## CONCLUSION

Applying a portion of the recommended nitrogen rate later in the season did not significantly affect the agronomic aspects of the leaf. Research in 2012 and 2013 indicated that yield, quality, and value would not be affected using the proposed treatments discussed in this study. When considering the weather for 2012 and 2013 it is important to evaluate/predict what would have happened in a year where rainfall totals were closer to average or even below average.

Research at the two environments in 2012 and the three environments in 2013 provided excellent representation of the variety of growing conditions that were seen across the state of North Carolina. Rainfall was above average late in the season for 2012 and above average early in the season in 2013 likely affectingnitrogen in soil solution due to leaching. Had less rainfall been recorded it is probable that differences in yield and quality may have been likely, therefore possibly affecting value per area.

Total alkaloids and reducing sugar percentages vary from year to year. The ratio between the two will likely be similar with slightly higher total alkaloids if less rainfall was recorded. Leaf nitrogen content at layby and topping would produce similar results as the crop was never put into a nitrogen deficient state, therefore uptake would be closely related to the data shown.

Using a liquid fertilizer source allowed easy of application and reduced grower inputs. This choice in fertilizer and application method is easily obtainable and useable by the majority of growers in tobacco producing regions of the US.Current nitrogen rate recommendations appear to be adequate for optimum yield and quality. Weather played a major role in this study. Had less rainfall occurred some of the late season applications may have resulted in a decreased quality leaf. Further research is still needed to determine if a late season application would not harm quality due to delayed ripening of the leaf.

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**Table 1.Soil characteristics, cultivar, transplant date, recommended nitrogen rate, and potassium rate at each environment.**

| Environment | Soil Series | Soil pH | Variety | Transplant date | Base nitrogen rate | $K_2O$ |
|---|---|---|---|---|---|---|
| | | | | | ----------Kg/ha---------- | |
| BBTRS-12[1] | Goldsboro loamy sand, *Fine-loamy, siliceous, subactive, thermic AquicPaleudults* | 6.0 | NC 71 | 4/18/2012 | 74 | 87 |
| OTRS-12[2] | Helena sandy loam, *Fine, mixed, semiactive, thermic AquicHapludults* | 5.9 | CC 35 | 5/14/2012 | 80 | 148 |
| BBTRS-13[3] | Goldsboro loamy sand, *Fine-loamy, siliceous, subactive, thermic AquicPaleudults* | 5.7 | NC 196 | 4/17/2013 | 80 | 111 |
| OTRS-13[3] | Helena sandy loam, *Fine, mixed, semiactive, thermic AquicHapludults* | 6.0 | CC 27 | 5/10/2013 | 80 | 84 |
| UCPRS-13[5] | Norfolk Loamy Sand *Fine-loamy, kaolinitic, thermic TypicKandiudults* | 5.9 | NC 196 | 4/24/2013 | 80 | 94 |

[1]BBTRS-12 Whiteville, NC; [2]OTRS-12 Oxford, NC; [3]BBTRS-13 Whiteville, NC; [4]OTRS-13 Oxford, NC; [5]UCPRS-13 Rocky Mount, NC

**Table 2.Rainfall by month at each environment.**

| Month | BBTRS-12[1] | OTRS-12[2] | BBTRS-13[3] | OTRS-13[4] | UCPRS-13[5] |
|---|---|---|---|---|---|
| | ----------cm---------- | | | | |
| **April** | 5.9 | 9.9 | 5.8 | 11.6 | 6.8 |
| **May** | 22.8 | 15.6 | 57.5 | 11.6 | 7.3 |
| **June** | 5.8 | 5.7 | 65.5 | 26.3 | 25.3 |
| **July** | 20.6 | 28.1 | 31.3 | 24.1 | 12.5 |
| **August** | 36.5 | 16.5 | 16.9 | 10.7 | 11.5 |
| **September** | 10.6 | 13.8 | 12 | 4.9 | 12.3 |
| **October** | 11.4 | 6.3 | 2.7 | 5.9 | 8.3 |
| **Total** | **113.6** | **95.9** | **191.7** | **95.1** | **84** |
| **Average** | **80** | **70** | **80** | **70** | **70** |
| **Percent above average** | **41%** | **37%** | **140%** | **37%** | **20%** |

[1]BBTRS-12 Whiteville, NC; [2]OTRS-12 Oxford, NC; [3]BBTRS-13 Whiteville, NC; [4]OTRS-13 Oxford, NC; [5]UCPRS-13 Rocky Mount, NC

*State climate office of North Carolina-CRONOS database.* 2013. Retrieved February 18, 2014 from http://www.nc-climate.ncsu.edu/cronos

**Table 3.Nitrogen application timing and amount applied relative to recommended nitrogen rates at each environment.**

| Treatment Number | ----------Nitrogen Application Timing---------- | | | | |
|---|---|---|---|---|---|
| | At Transplanting | 2 Weeks after transplanting[1] | At layby[2] | 2 Weeks after layby[3] | At Topping |
| **1 (Control)** | 50% | - | 50% | - | - |
| **2** | 50% | 25% | - | - | - |
| **3** | 25% | 25% | 25% | 25% | - |
| **4** | 25% | 25% | 25% | - | - |
| **5** | 25% | - | 25% | 25% | 25% |
| **6** | 25% | - | 25% | 25% | - |
| **7** | 25% | 25% | - | - | 50% |
| **8** | 25% | - | 25% | - | 25% |
| **9** | 50% | - | 50% | - | 25% |
| **10** | 50% | - | 25% | - | 25% |

*Treatments were the same across all environments
[1]2 weeks after transplanting plants were approximately 20 cm tall
[2]At layby plants were approximately 38 cm tall
[3]2 weeks after layby plants were 50 cm tall

**Table 4. P values for yield, quality, value, total alkaloids, reducing sugars, leaf nitrogen content at layby, leaf nitrogen content at topping, leaf nitrogen content of cured leaf, and SPAD measurements.**

| Variable | Pr>F Env* Rate/Timing | Pr>F Rate/Timing |
|---|---|---|
| **Yield** | 0.7037 | 0.3448 |
| **Quality** | 0.9803 | 0.5747 |
| **Value** | 0.8286 | 0.3988 |
| **Total Alkaloids** | 0.0618 | <0.0001 |
| **Total Reducing Sugars BBTRS-12[1]** | --- | 0.1201 |
| **Total Reducing Sugars OTRS-12[2]** | --- | 0.0026 |
| **Total Reducing Sugars BBTRS-13[3]** | --- | 0.3415 |
| **Total Reducing Sugars OTRS-13[4]** | --- | 0.574 |
| **Total Reducing Sugars UCPRS-13[5]** | --- | 0.1585 |
| **Leaf nitrogen content at layby** | 0.1454 | 0.0038 |
| **Leaf nitrogen content at topping BBTRS-12[1]** | --- | 0.0917 |
| **Leaf nitrogen content at topping OTRS-12[2]** | --- | 0.0250 |
| **Leaf nitrogen content at topping BBTRS-13[3]** | --- | 0.0039 |
| **Leaf nitrogen content at topping OTRS-13[4]** | --- | 0.8992 |
| **Leaf nitrogen content at topping UCPRS-13[5]** | --- | 0.0090 |
| **Leaf nitrogen content of cured leaf** | | |
| **SPAD BBTRS-12** | --- | 0.0558 |
| **SPAD OTRS-12** | --- | 0.0461 |
| **SPAD BBTRS-13** | --- | 0.0012 |
| **SPAD OTRS-13** | --- | 0.8946 |
| **SPAD UCPRS-13** | --- | 0.0187 |

[1]BBTRS-12 Whiteville, NC
[2]OTRS-12 Oxford, NC
[3]BBTRS-13 Whiteville, NC
[4]OTRS-13 Oxford, NC
[5]UCPRS-13 Rocky Mount, NC

**Table 5. Yield, quality, and value response to nitrogen rate and timing combined over environments.**

| Treatment Number | ----------Nitrogen Application Timing---------- | | | | | Yield Kg/ha | Quality Index[1] | Value $/ha |
|---|---|---|---|---|---|---|---|---|
| | At Transplanting | 2 WAT | At layby | 2 WALayby | At Topping | | | |
| **1 (Control)** | 50% | - | 50% | - | - | 2,875 a | 80 a | 10,492 a |
| **2** | 50% | 25% | - | - | - | 2,901 a | 82 a | 10,808 a |
| **3** | 25% | 25% | 25% | 25% | - | 2,759 a | 82 a | 10,484 a |
| **4** | 25% | 25% | 25% | - | - | 2,779 a | 83 a | 10,731 a |
| **5** | 25% | - | 25% | 25% | 25% | 3,104 a | 80 a | 11,444 a |
| **6** | 25% | - | 25% | 25% | - | 2,827 a | 82 a | 10,600 a |
| **7** | 25% | 25% | - | - | 50% | 2,771 a | 78 a | 9,824 a |
| **8** | 25% | - | 25% | - | 25% | 2,787 a | 79 a | 9,829 a |
| **9** | 50% | - | 50% | - | 25% | 2,847 a | 78 a | 10,007 a |
| **10** | 50% | - | 25% | - | 25% | 2,948 a | 84 a | 11,368 a |

*Treatments followed by the same letter within a column do not represent significant differences based on ANOVA analysis.
[1]Bowman *et al.* Revised North Carolina grade index for flue-cured tobacco. 1988

**Table 6. Total alkaloids, leaf nitrogen content at layby, and SPAD measurement responses to nitrogen rate and timing combined over environments.**

| Treatment Number | ----------Nitrogen Application Timing---------- | | | | | Total alkaloids | Nitrogen content at layby | SPAD[1] |
|---|---|---|---|---|---|---|---|---|
| | At Transplanting | 2 WAT | At layby | 2 WALayby | At Topping | ----------%---------- | | |
| **1 (Control)** | 50% | - | 50% | - | - | 2.65cde | 4.34 ab | 10,492 a |
| **2** | 50% | 25% | - | - | - | 2.49 ef | 4.61 a | 10,808 a |
| **3** | 25% | 25% | 25% | 25% | - | 2.54def | 4.15 bc | 10,484 a |
| **4** | 25% | 25% | 25% | - | - | 2.41 f | 4.17 bc | 10,731 a |
| **5** | 25% | - | 25% | 25% | 25% | 2.76abc | 4.10 bc | 11,444 a |
| **6** | 25% | - | 25% | 25% | - | 2.43 f | 4.00 c | 10,600 a |
| **7** | 25% | 25% | - | - | 50% | 2.85 ab | 4.23 bc | 9,824 a |
| **8** | 25% | - | 25% | - | 25% | 2.56cdef | 4.04 bc | 9,829 a |
| **9** | 50% | - | 50% | - | 25% | 2.95 a | 4.29 bc | 10,007 a |
| **10** | 50% | - | 25% | - | 25% | 2.72bcd | 4.34 ab | 11,368 a |

*Treatments followed by the same letter within a column do not represent significant differences based on ANOVA analysis.
[1]SPAD, *Technical bulletin.* Minolta Camera Co., Ltd. Osaka, Japan

**Table 7.Total reducing sugars response to nitrogen rate and timing by environment.**

| Treatment | ----------Nitrogen Application Timing---------- | | | | | BBTRS-12 | OTRS-12 | BBTRS-13 | OTRS-13 | UCPRS-13 |
|---|---|---|---|---|---|---|---|---|---|---|
| | At Transplanting | 2 WAT | At layby | 2 WALayby | At Topping | ----------% Reducing Sugars---------- | | | | |
| **1 (Control)** | 50% | - | 50% | - | - | 14.04 a | 13.43 bcde | 20.22 a | 16.78 a | 15.97 a |
| **2** | 50% | 25% | - | - | - | 16.19 a | 12.65 bcde | 21.82 a | 15.75 a | 19.18 a |
| **3** | 25% | 25% | 25% | 25% | - | 15.13 a | 14.75 abc | 19.37 a | 14.38 a | 15.59 a |
| **4** | 25% | 25% | 25% | - | - | 16.95 a | 16.085 ab | 19.82 a | 15.82 a | 18.00 a |
| **5** | 25% | - | 25% | 25% | 25% | 12.97 a | 12.09 cde | 18.71 a | 15.20 a | 16.52 a |
| **6** | 25% | - | 25% | 25% | - | 16.86 a | 17.54 a | 20.16 a | 14.71 a | 17.89 a |
| **7** | 25% | 25% | - | - | 50% | 14.23 a | 11.07 de | 19.19 a | 13.85 a | 18.00 a |
| **8** | 25% | - | 25% | - | 25% | 15.97 a | 10.69 ed | 21.55 a | 16.38 a | 17.89 a |
| **9** | 50% | - | 50% | - | 25% | 13.93 a | 9.97 e | 19.14 a | 11.60 a | 15.41 a |
| **10** | 50% | - | 25% | - | 25% | 15.00 a | 13.92 bcd | 21.47 a | 12.45 a | 16.09 a |

*Treatments followed by the same letter within a column do not represent significant differences based on ANOVA analysis.

**Table 8.Leaf nitrogen content at topping in response to nitrogen rate and timing by environment.**

| Treatment | ----------Nitrogen Application Timing---------- | | | | | BBTRS-12 | OTRS-12 | BBTRS-13 | OTRS-13 | UCPRS-13 |
|---|---|---|---|---|---|---|---|---|---|---|
| | At Transplanting | 2 WAT | At layby | 2 WALayby | At Topping | ----------% Nitrogen at Topping---------- | | | | |
| **1 (Control)** | 50% | - | 50% | - | - | 2.11a | 3.88 abc | 1.54 cd | 2.93 a | 2.48 a |
| **2** | 50% | 25% | - | - | - | 1.87 a | 4.19 ab | 1.43 d | 2.84 a | 1.79 c |
| **3** | 25% | 25% | 25% | 25% | - | 1.91 a | 4.30 a | 1.73 cd | 3.04 a | 2.00 bc |
| **4** | 25% | 25% | 25% | - | - | 1.85 a | 3.61 c | 1.52 cd | 2.87 a | 2.07 bc |
| **5** | 25% | - | 25% | 25% | 25% | 2.15 a | 3.50 c | 2.56 a | 2.85 a | 2.03 bc |
| **6** | 25% | - | 25% | 25% | - | 1.99 a | 3.73 bc | 1.97 bc | 2.91 a | 2.05 bc |
| **7** | 25% | 25% | - | - | 50% | 2.57 a | 3.79 bc | 2.26 ab | 2.76 a | 1.83 c |
| **8** | 25% | - | 25% | - | 25% | 1.84 a | 4.13 ab | 1.72 cd | 2.88 a | 2.33 ab |
| **9** | 50% | - | 50% | - | 25% | 2.18 a | 4.20 ab | 1.75 cd | 3.16 a | 2.46 a |
| **10** | 50% | - | 25% | - | 25% | 1.97 a | 3.83 abc | 1.76 cd | 2.86 a | 2.03 bc |

*Treatments followed by the same letter within a column do not represent significant differences based on ANOVA

**Table 9.SPAD measurements in response to nitrogen rate and timing by environment.**

| Treatment | ----------Nitrogen Application Timing---------- | | | | | BBTRS-12 | OTRS-12 | BBTRS-13 | OTRS-13 | UCPRS-13 |
|---|---|---|---|---|---|---|---|---|---|---|
| | At Transplanting | 2 WAT | At layby | 2 WALayby | At Topping | ----------SPAD[1] Value---------- | | | | |
| **1 (Control)** | 50% | - | 50% | - | - | 43.43 a | 45.75 ab | 31.47 de | 46.90 a | 47.17 ab |
| **2** | 50% | 25% | - | - | - | 38.83 b | 44.26 abc | 28.23 e | 44.90 a | 41.63 c |
| **3** | 25% | 25% | 25% | 25% | - | 41.63 ab | 44.05 abc | 39.37 ab | 46.90 a | 41.93 c |
| **4** | 25% | 25% | 25% | - | - | 39.93 ab | 41.80 c | 29.30 e | 45.00 a | 41.63 c |
| **5** | 25% | - | 25% | 25% | 25% | 43.90 a | 45.40 ab | 42.37 a | 44.68 a | 42.03 c |
| **6** | 25% | - | 25% | 25% | - | 38.30 b | 43.38 bc | 38.63 abc | 44.20 a | 42.30 bc |
| **7** | 25% | 25% | - | - | 50% | 43.53 a | 43.18 bc | 33.03 cde | 45.20 a | 40.47 c |
| **8** | 25% | - | 25% | - | 25% | 38.13 b | 45.33 ab | 31.83 ed | 45.35 a | 44.40 bc |
| **9** | 50% | - | 50% | - | 25% | 38.40 b | 45.68 ab | 35.47 bcd | 46.20 a | 50.00 a |
| **10** | 50% | - | 25% | - | 25% | 40.67 ab | 46.63 a | 38.93 abc | 44.35 a | 43.40 bc |

*Treatments followed by the same letter within a column do not represent significant differences based on ANOVA
[1]SPAD, *Technical bulletin.* Minolta Camera Co., Ltd. Osaka, Japan

# CHAPTER THREE

# NITROGEN APPLICATION RATE AND MEITHOD EVALUATION ON YIELD, QUALITY, AND CHEMICAL COMPONENTS OF FLUE-CURED TOBACCO (*Nicotianatabacum*L.)

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**ABSTRACT**

Research was conducted at two environments in 2012 and three environments in 2013 to determine the effect of nitrogen application rate and method on yield, quality, and leaf chemistry of flue-cured tobacco. Treatments included different of nitrogen rates and method of application.Nitrogen rates above, at, and below recommended levels for a specific location were split into two to four application timings depending on the treatment until topping.Nitrogen was either soil applied or foliar applied through stalk run-down at the topping stage of plant growth. Nitrogen applications made before topping were applied directly to the soil. Urea ammonium nitrate (UAN) 28% was the nitrogen source that was used across all treatments. Yield data were taken and leaf quality was determined by a USDA grade. Crop value per hectare was determined using the quality grade, which has a set market price for specific grades.

Tissue samples were taken at layby (when plants are roughly 38 cm high), at topping (approximately 60-70 days after transplant), and after curing to evaluate total nitrogen content of the leaf at the specified growth stages. Tissue samples of the cured leaf were analyzed for both total alkaloids and reducing sugars. All data were subjected to analysis of variance (ANOVA) using PROC GLM procedure in SAS. Treatment means were separated using Fisher's Protected LSD test at $p \leq 0.05$

Method of application significantly affected yield with soil applied nitrogen treatments yielding higher than foliar applied nitrogen treatments. Leaf quality was affected by nitrogen timing and method. Likely due to the significant effects ofapplication methodand

application timing value per area was significantly affected by application timing and method. Total alkaloid levels were unaffected by timing or method. Reducing sugar levels had a significant nitrogen application timing effect.Reducing sugar levels were lowest for plots with less nitrogen applied at topping. Leaf nitrogen content at layby had a significant application timing affect as higher leaf nitrogen came from plots with higher amounts of nitrogen applied early in the season. Leaf nitrogen content at topping from foliar applied nitrogen was significantly higher than leaf nitrogen content of soil applied nitrogen. Method of application affected SPAD measurements. SPAD measurements were significantly higher for foliar applied nitrogen than for soil applied nitrogen. The multiple variables with significantnitrogen method application p-values suggest that method of application was a factor in affecting the various data received from the research.

Rainfall played a major role in the outcome of this research. In this research late season nitrogen application did not affect yield, quality and leaf chemistry. If similar precipitation amounts are not observed, results may be different.

## NOMENCLATURE

*Nicotianatabacum*L., tobacco, urea ammonium nitrate.

## KEY WORDS

Nitrogen, nitrogen rate, nitrogen method, UAN, application method.

## INTRODUCTION

### Function and Role of Nitrogen in Flue-Cured Tobacco

Nitrogen can be considered the most important macronutrient in flue-cured tobacco (*Nicotianatabacum* L.) production. Appropriate levels are needed to produce a high yielding, quality leaf, with appropriate aroma and smoke flavor (McCants and Woltz, 1967). Flue-cured tobacco will accumulate approximately fifty percent of the total nitrogen absorbed in a season within five weeks of transplanting with the majority of the nitrogen being absorbed between the fourth and fifth week. The remainder will be accumulated within eight to nine weeks after transplanting (Raper and McCants, 1966). Nitrogen must be applied in adequate amounts and be available to the plant during the essential stages of growth to have the greatest influence on chemical properties of the leaf (Chaplin and Miner, 1980). Ripeness of the leaf is influenced by nitrogen in the season and ripeness is correlated with leaf qulaity. Excess nitrogen will delay ripeness and cause the leaf to become “dark brown to black in color and dry and chaffy” (McCantz and Woltz, 1967). Deficiencies of nitrogen can cause immature tobacco leaves to appear ripe if only the color of the leaf is being evaluated.

However, the cured leaves of nitrogen deficient tobacco will be pale in color and lack both physical and chemical properties that are common in quality tobacco leaf and will not cure properly (McCantz and Woltz, 1967). Maturity of the tobacco plant is correlated with age of the plant while ripeness is a factor of nitrogen attenuation as the growing season progresses (Fisher, 2013). Also, an unripe leaf may be mature, but due to excess nitrogen may not be considered ripe therefore, if harvested will not produce a quality leaf. Due to the unique harvesting of the vegetative portion of the tobacco plant, starting from the base moving towards the top portion of the plant, leaves at different stalk positions will be at different physiological growth stages at any specific point in time (McCantz and Woltz, 1967). It is important that as the plant reaches its maximum leaf area that the nitrogen in the system starts to decline for proper leaf quality (McCantz and Woltz, 1967). This decline in nitrogen after maximum leaf area is reached allows for the harvest of properly ripened leaves without losing yield potential.

The correct proportion of reducing sugars to total alkaloids is important for the taste and aroma of tobacco (Tso, 1990b). Nicotine is one of the major alkaloids that is desirable in tobacco (Collins and Hawks, 1993). Therefore, the proper rate of nitrogen is important to produce reducing sugars and nicotine ratio that is about 6 to 8:1 (Flower, 1999). Under fertilized tobacco or tobacco that has been subject to leaching have higher ratios of reducing sugars to total alkaloids. Over fertilized tobacco or tobacco that has been subject to drought have lower ratios of reducing sugars to total alkaloids (Flower, 1999). Nicotine and other

chemical constituents to smoke characteristics are produced through the sikimate pathway as discussed earlier in the chapter (Lea, 2001).

Flue-cured tobacco is generally grown on sandy and sandy loam soils (Flower, 1999). With this variation in soil types, nitrogen recommendations vary depending on the soil type. Sandier soils may require 80 pounds of nitrogen per acre while soils with a greater percentage of clay may only require 50 pounds of nitrogen per acre (Collins and Hawks, 1993). Current recommendations still follow these same rates to achieve optimum quality and yield (Vann and Smith, 2014). Nitrogen is the second most absorbed nutrient next to potassium in flue-cured tobacco but is the key nutrient in tobacco fertilization (Flower, 1999). Since the soils where flue-cured tobacco is grown are typically sandier and well drained, organic matter is typically low therefore soil nitrogen reserves are usually less, adding to the importance of proper nitrogen application rates (Peedin, 1999). Tobacco that has received excessive rainfall will experience soils with heavily leached nitrogen and can result in high sugar, low nicotine tobacco (Peedin, 1999). Drought stressed tobacco is just the opposite with low sugar and high nicotine content (Peedin, 1999). Neither of which are desirable in a quality crop. Due to the leaching potential of nitrate many growers have changed what was considered common production practices in the past decade. Banding of nitrogen is now common in comparison to a broadcast application of fertilizer (Tso, 1990a). The use of urea ammonium nitrate (UAN) as a portion/entire amount of the required nitrogen has also become a popular practice (Vann and Smith, 2014). These practices reduce the chances of leaching which aid in the production of quality leaf. Transitioning from a granular

blended fertilizer to a UAN product can save the grower potentially 40-45% on his/her fertilizer cost (Vann and Smith, 2014).

The form of nitrogen needed in tobacco production has long been debated. Early work done by McCants and Woltz (1963 and 1967) and many others indicated that when nitrate is less than 30 percent of the total nitrogen source applied then reduced growth can occur (Peedin, 1999). Parker (2009) verified that nitrogen source did not affect yield, grade index, value, total alkaloids, total reducing sugars, or leaf color (Parker, 2009). Switching to either all or a portion of the nitrogen applied from UAN can help the grower reduce nitrogen loss in a cropping system and decrease the input cost for the crop without compromising yield or quality.

Current nitrogen rates applied to the tobacco crop are based on soil type, cultural practices, and previous field history (Vann and Smith, 2014). With research previously conducted on fertility and nitrogen source the next step for current production was to determine if it was possible to split nitrogen applications into multiple applications. Applying nitrogen in more than two applications would allow growers to base nitrogen rates on current growing conditions in conjunction with field history. With nitrogen availability being so closely correlated to weather conditions, and tobacco leaf quality being directly related to nitrogen availability, a new nitrogen regiment of more than two applications in a given year could assist the grower in producing quality tobacco without reducing yield. The present research was conducted to determine if splitting nitrogen applications into more than two

applications was beneficial for proper plant growth and to determine when the late season threshold for application occurs.

Application timing of nitrogen is vital to proper curing of the tobacco leaf and was evaluated individually in a separate study. Another important aspect of late season nitrogen application is the method in which the nitrogen should be applied. The possibility of a late season application is practical using high clearance sprayers and the use of UAN.By using high clearance sprayersgrowers can either apply liquid nitrogen to the soil surface using drop-lines or using the sucker control system let the product run down the stalk. There is currently no information on the efficiency of either of these methods.Therefore, research was conducted to determine if there are any differences between the two methods of applications. The study evaluated whether method of application affected response to nitrogen, if there is a time in the growing season that is too late to apply nitrogen, and could a late season application be beneficial in barn utilization so that growers can get the most benefit out of their current infrastructure.

## METHODS AND MATERIALS

### Field Procedures

Research was conducted at the Border Belt Tobacco Research Station (BBTRS) near Whiteville, NC and the Oxford Tobacco Research Station (OTRS) in Oxford, NC in 2012. An additional location at the Upper Coastal Plains Research Station (UCPRS) near Rocky Mount NC in 2013. Given that each location within a year and locations from year to year

behave independently of the other locations, a specific year plus the corresponding location will be referred to as an environment. Tobacco was produced using suggested practices according to North Carolina Cooperative Extension Service, based on specific growing environments with the exception of experimental treatments (Fisher, 2014). After curing, leaves were weighed to determine yield and assigned an USDA government grade. Government grades were based on leaf maturity and ripeness. The grades are assigned a value that is associated with a grade index value (Bowman et al., 1988).

Treatments consisted of nitrogen applications made in different combinations of timing and rate. Timings of nitrogen applications were based on growth stages of the plant. The lay-by treatment (approximately 38 cm height) and at topping (approximately 120 cm height) treatments were approximately four and eight weeks after transplant, respectively. The controls for the study consisted of 50% of the recommended nitrogen applied at transplanting and 50% of the recommended nitrogen applied at layby and a control that had 50% of the recommended nitrogen applied at transplanting and then 25% applied two weeks after transplanting. The controls were to simulate what the majority of tobacco growers are currently using and also give a comparison for what would be expected in a year where less rainfall is received and the grower only would apply a portion of the needed nitrogen. All treatments had 28% UAN applied to the soil surface using either a TG 5 or TG 3 full cone nozzle depending on the rate of nitrogen that was required. Treatments were applied using $CO_2$ backpack sprayer. Pressure ranged from 100-175 kPa depending on rate and nozzle size. Treatments were replicated four times and arranged in a randomized complete block design.

There were four rows per plot that were 12.2 meters long and 4.9 m wide. Plant spacing between individual plants within the rows was 0.55 m in all environments. Tissue sampling and leaf harvest were performed on the two center rows. The outside two rows served as border rows between treatments. Due to extreme rainfall in the 2013, season we did not evaluate the study at the Boarder Belt Tobacco Research Station.

Cultivar selection was dependent upon the specific environment and are listed in Table 10. Tissue samples were taken at three time intervals, at layby, at topping, and from the cured leaf. Layby is approximately four weeks after transplanting and 38 cm tall and topping is approximately eight weeks after transplanting and 120 cm tall. Total nitrogen content of the leaf was assessed from all three timing intervals. The green leaf tissue (layby and at topping) samples were taken from either the third or fourth leaf from the apex of the plant. The leaves measured approximately 10 cm wide and 15 cm long and were taken from approximately five plants within each plot. After sampling, the tissue was dried, ground, and sent to the North Carolina State University Tobacco Analytical Services Lab for total leaf nitrogen content analysis. Both layby and at-topping samples were taken approximately one week after the corresponding layby or at topping nitrogen treatments were applied. Chlorophyll was estimated using a Konica Minolta SPAD-502 meter and taken from this same leaf position on the same day that leaf samples were taken for the “at-topping” green leaf tissue samples. In each plot ten leaves were measured using a SPAD meter and then averaged across the entire plot. After curing, a composite sample across stalk positions of cured leaf was taken to be analyzed for nitrogen, total alkaloids, and reducing sugars.

**Field Conditions**

Field conditions (cultivar, transplant date, pH, base nitrogen rates, and $K_2O$ rate) (Table 10) varied by environment as well as monthly rainfall (Table 11). Urea ammonium nitrate (28%) supplied all nitrogen for treatments either a banded or broadcast application of K-Mag (0-0-22) as the potassium source which were based on soil test results for potassium. No additional phosphorus was added as all soil reports showed high to very high P-index values therefore no additional P is recommended (Vann and Smith, 2014). The K-Mag also supplied the magnesium (10.8%) and sulfur (22%) needed for proper plant growth (Fisher, 2014).

Nitrogen was applied to each plot depending on treatment and there were a total of ten treatments including the control (Table 12). Nitrogen application started at transplanting and was completed prior to or at topping depending on the treatment. Possible application timings evaluated were at planting, at 15 cm plant height, at 38 cm plant height (layby), at 60 cm plant height and at 120 cm plant height (topping). Timings corresponded with approximately 0, 2, 4, 6, and 8 weeks after transplanting. Rates of nitrogen applied were determined by the individual environments. Within each environment, rates of nitrogen applied were 75% of the total recommended nitrogen rate (treatments 2, 4, 6, 8), 100% of the recommended nitrogen rate (treatments 1, 3, 5, 7, 10), or 125% of the recommended rate (treatment 9), (Table 12). The research stations established nitrogen rate was used to determine the required amount for each treatment. Nitrogen was soil applied using a simulated soil directed application technique and was then incorporated with a Danish tine

rolling cultivator. The “at topping” application was done to simulate drop nozzles used by high clearance sprayers to apply nitrogen to the soil surface or directly to the leaf foliage depending on the treatment (Table 12).Treatments were arranged in a three nitrogen rate by two application method factorial with a randomized complete block design including appropriate controls as mentioned.

The soil applied method of application was accomplished using a simulated soil directed application technique. It was done to mirror the use of a drop line on a high clearance sprayer. Nitrogen was applied as close to the base of the plant as possible without causing leaf damage. The stalk run-down method of application was to simulate the use of a sucker control application equipment. Urea ammonium nitrate was mixed with water and applied over the top of the plant at a solution volume of 468 liters per hectare.Amount of UAN added to the spray solution was dependent on the rate of nitrogen that needed to be applied. The solutioncontained 15 liters of 28% UAN to apply 20 kilograms of nitrogen per hectare for eachrequired application. Treatments that called for more than 25% of the total nitrogen to be applied as stalk run-down were split into two spray applications. This double application was due to the observed phytotoxicity to the plant.

**Analytical Procedures**

*Nitrogen.*Total leaf nitrogen content was analyzed using the procedure of Nelson and Sommers 1973) in 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 the sample size was ground to < 80-mesh material to improve nitrogen recovery and shorten measurement time for each sample (Nelson and Sommers, 1973).

*Total Alkaloidsand Reducing Sugars*. Total alkaloids and Reducing sugars were analyzed using Perkin-Elmer Autosystem XL Gas Chromatograph procedure in the North Carolina State University Tobacco Analytical Services Laboratory. This procedure determines total alkaloids based on chromatographic peak response at the retention times of alkaloid standards (Lewis et al., 2012).

*SPAD-502 Measurements.* SPAD-502 measurements were taken using Minolta chlorophyll meter. SPAD-502 measurements are relative chlorophyll measurements that nondestructively measure red region transmittance peak of chlorophyll a and b (around 660 nm) of the leaf and calculate a numerical value (SPAD value). This value is proportional to the amount of chlorophyll present in the leaf (Technical Bulletin, 1989). Greener leaves can then be correlated to higher leaf nitrogen content (Yoderand Pettigrew-Crosby, 1995).

*Statistical Analysis.* Data for crop yield, quality, value, percent nitrogen at layby, percent nitrogen at topping, percent nitrogen of cured leaf, percent total alkaloids, percent reducing sugars, and SPAD measurements were analyzed in SAS version 9.3. All parameters listed were subjected to an analysis of variance (ANOVA) using the PROC GLM procedure and treatment means were separated in accordance with Fisher's Protected LSD test with a p-value set at $p \leq 0.05$.

## RESULTS AND DISSCUSION

Interactions between environment and main effects varied across the components that were evaluated. Each component will be reviewed and discussed based on specific significant interactions that were noted. All measured variables had some significant p-value for one or more of the dependent variables except for the total alkaloid percentages (Table 13).

### Treatments compared to Controls

The study was evaluated as a randomized complete block design to compare treatments to the controls and then as a 3 x 2 factorial to evaluate differences between application timing and differences between application method. Yield and value for treatments in factorial were different the controls. Quality did have an environment interaction and significant differences when compared to the controls at the Boarder Belt Tobacco Research Station in 2012. The late season nitrogen application may have delayed ripening causing a decrease in quality. The controls had the lowest alkaloid levels which could be contributed to the lack of a late season nitrogen application. Reducing sugars were lower in the controls than with one factorial treatment. Treatments with foliar applied nitrogen had higher nitrogen content at topping than the controls. Nitrogen content at layby and SPAD measurements were not different from controls.

**Yield, Quality, and Value**

*Yield.*There was no environment by treatment interaction for yield therefore means were combined across all environments (Table 13). The timing by method interaction was not significant (Table 13). Nitrogen application method was significant (Table 13). Soil applied nitrogen yielded 122 kg/ha higher than stalk run-down applied nitrogen(Table 14). This could have two possible explanations. First, the amount of effective rooting zone the nitrogen solution comes into contact with is greater. When the solution is applied to the soil surface leading to a greater possibility for root interception. When the stalk run-down method is used all of the solution moves to the base of the plant, this area is much less when compared to a continuous band placed on the soil surface. Also the volume of solution being applied with the stalk run-down is approximately 30 mL per plant (based on 468 L/ha). With little to no nutrient absorption through the leaf tissue the critical placement of the solution is at the base of the plant. Any solution that stays on the leaf surface and dries will not reach the effective rooting zone unless it is facilitated with a wash off rainfall event (naturally occurring or irrigation). When this additional water volume is added there is an obviously dilution of the solution, possibly decreasing the amount of nitrogen that will stay in the effective rooting zone.

Excess rainfall occurred in 2013 (Table 11) but there was a lack of effect on yield across all environments. This is likely due to when the majority of the rainfall occurred. Many of the treatments were applied after the rain was recorded therefore if nitrogen did leach from the soil profile it was replaced by late season nitrogen applications. Where

nitrogen deficiencies were observed there was no long term effect on yield of cured leaf. As noted earlier we did not include data from the Border Belt Tobacco Research Station due to the entire test being exposed to excess standing water causing yield loss and/or plant damage. Excess rainfall late in the season in 2012 may have aided in the leaching of nitrogen from the soil profile. The leaching in 2012 likely contributed to reduced nitrogen availability and timely ripening of the leaf. Had less leaching taken place results may have been different.

*Grade Index.*There was no environment by treatment interactiontherefore means were combined across all environments (Table 13) A significant timing by method interaction was observed so individual treatments were analyzed (Table 15).No consistent trends were found among treatments.

It has been noted that increasing nitrogen rates above the recommended nitrogen amount can lead to decreased quality and a delay in ripening (Felipe and Long, 1988). However, this was not observed in the study as it was not a direct correlation to excess nitrogen that affected quality. It is plausible that the excess rainfall in both years leached nitrogen from the soil profile and therefore ripening of the crop was possible. In 2012, with later season rainfall any excess nitrogen in the profile would have likely leached from the effective rooting zone. In 2013 there was likely little to no nitrogen in the profile after early season precipitation due to leaching, therefore adding a late season nitrogen application did not add excess nitrogen to the soil profile. These later season applications in 2013 would be more accurately described as leaching adjustment though this was not the primary intention of the study. It is likely for treatments that are applying 50% of the recommended nitrogen at

topping that there would be a delay in ripening with less rainfall. Assumptions on potential leaching of nitrogen are based on previous research regarding soil profile nitrogen and excess rainfall (Fisher, 2014).Precaution should be used when applying nitrogen as a stalk run-down application as too much nitrogen applied at one time can cause leaf necrosis. In this study, rates of nitrogen above 25% of the required nitrogen caused leaf damage. Fifteen liters of 28% UAN combined with water to total a 468 liter per hectare spray solution was safe for foliar application in the majority of the test. Thin, stressed tobacco did have some leaf damage from this rate but on healthy growing plants this rate proved to be effective in supplying the needed nutrient without causing leaf damage.

*Crop Value.*With the significant effects of method of application for yield and the significant interaction in the quality index it not surprising that crop value per hectare was affected. A significant environment by timing by method interaction and a significant timing by method interaction were observed (Table 13). Individual environments were analyzed as well as the individual treatment means. The only environment with a significant treatment interaction was the Upper Coastal Plains Research Station in 2013 (Table 16). The three treatments that received the highest value per hectare were treatments that had the least amount of nitrogen applied. Of these three treatments both methods of application were used.Therefore, no correlation between value and method of application can be assumed directly. The increase in yield from the soil applied nitrogen method when evaluating yield combined with the differences in quality index likely led to differences in crop value.

**Chemical Characteristics**

*Total Alkaloids.*No response was observed for total alkaloid levels (Table 13.) There was no significant environmental interaction or any main effects. Due to the absorption of nitrogen through the roots method of application did not play an important role.All treatment averages for total alkaloids fell within the percentage range of 1,500 tobacco cultivars that were evaluated at a single environment grown under similar cultural practices (Chaplin, 1980).

*Reducing Sugars.*No significant environment interaction was observed for the reducing sugar percentage (Table 13) so environments were combined and evaluated. There was a significant main effect for nitrogen timing (Table 13).Treatments that had a portion of the recommended nitrogen applied at transplant were not significantly different from one another and had the highest reducing sugar percentages. For starch accumulation (reducing sugars) to begin nicotine and protein synthesis must have been terminated by the lack of plant nitrogen accumulation. This occurs in a nitrogen deficient system or when there is no more available nitrogen for plant uptake (Tso, 1990c). Treatments with a portion of their nitrogen applied at transplant had less nitrogen applied late in the season allowing for starch to accumulate earlier. This is the likely cause for the higher reducing sugar when compared to treatments that did not have nitrogen applied until two weeks after transplanting(Table 17).All treatment averages for total reducing sugar fell within the percentage range of 1,500 tobacco cultivars that were evaluated at a single environment grown under similar cultural practices (Chaplin, 1980).

*Leaf Nitrogen Content at Layby.*An environment by timing interactionwas observed (Table 13). Environments were evaluated independently due to this interaction. There was a significant main effect of timing at both the Oxford Tobacco Research Station and the Upper Coastal Plains Research Station in 2013 (Table 18). Differences observed are likely due to not all of the treatments had all of the nitrogen applied to those plots at the time of sampling. There were treatments that had 50% of the nitrogen applied after the layby stage, these treatments had less leaf nitrogen content than treatments that had already had 100% of the recommended rate applied when the samples were taken.

*Leaf Nitrogen Content at Topping.* A significant method by environment interaction was observed for the leaf nitrogen content at topping as well as a main effect for method of application (Table 13). Evaluation of each environment revealed significant main effects for method of application at the Oxford Tobacco Research Station in 2012 and 2013 (Table 19). The stalk run-down method had higher leaf nitrogen content at topping in both of the respective environments. Within each environment, percent leaf nitrogen content varied only slightly which is likely due to the lack of extreme nitrogen rate differences between the treatments. Research has shown that foliar up take of nitrogen in tobacco is not an effective means of uptake therefore, there is likely other possible reasons for this significant effect from the method of application. By letting the solution run to the base of the plant root interception with the nitrogen solution may be quicker. Since samples were taken on the same date for both application methods if stalk run-down was taken up sooner due to the placement of the solution then this may explain the higher leaf nitrogen content at

topping.Residue from the solution could have remained on the plant increasing the nitrogen percentage of the leaf as well.

*SPAD Measurements.* A significant method of application by environment interaction was observed for the SPAD measurements (Table 13). Evaluation of each environment revealed significant main effects for method of application at the Oxford Tobacco Research Station in 2012 and the Upper Coastal Plains Research Station in 2013 (Table 20). The stalk run-down application method for both of these environments represented higher SPAD measurements. With SPAD readings being indicative of chlorophyll content, higher measurements are likely correlated to the higher leaf nitrogen content at topping as both of these parameters were measured on the same day as well as from the same leaf position. The same stalk run-down method of application was significantly greater for SPAD measurements as well as leaf nitrogen content at topping. The majority of the plots with the highest SPAD readings also had the highest nitrogen content.

## CONCLUSION

Method of application and timing had significant effects on both agronomic and chemical aspects of the leaf. Research in 2012 and 2013 indicated that yield, quality, and value were affected by either the method of application or the interaction between method of application and timing of application. When considering the weather for 2012 and 2013 it is important to evaluate/predict what would have happened in a year where rainfall totals were closer to average.

Research at the two environments in 2012 and the twoenvironments in 2013 provided excellent representation of the variety of growing conditions that are seen across the state of North Carolina those years. Rainfall was above average late in the season for 2012 and above average early in the season in 2013. Both of the years weather conditions affect nitrogen in soil solution due to leaching. Had less rainfall been recorded it is probable that differences would be different.

Total alkaloids and reducing sugar percentages vary from year to year. The ratio between the two will likely be similar with slightly higher total alkaloids if less rainfall was recorded. Leaf nitrogen content at layby and topping would produce similar results as the crop was never put into a nitrogen deficient state, therefore uptake would be closely related to the data shown.

Using a liquid fertilizer source allowed ease of application and reduced grower inputs. This choice in fertilizer and application method is easily obtainable and useable by the majority of growers in tobacco producing regions of the US. Current application methods should be decided upon by the grower. Based on this research soil applied nitrogen out yielded stalk run-down nitrogen.Weather played a major role on the effects of the treatments. Had less rainfall occurred the late season application with 50% of the total nitrogen applied may have resulted in a decreased quality leaf. Further research is still needed to determine if late season method of application would not have a greater effect on quality then what was observed. Over the top applications should be used with caution adding no more than 20 kg/ha of nitrogen in a 468 L volume solution due to potential burning of the leaf. Even at this

rate precaution should be taken. When nitrogen and a water solution is sprayed a safer amount of product per acre would be 11-17 kg/ha of nitrogen per pass when mixed with 468 L/ha per pass.

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**Table 10.Soil characteristics, cultivars, transplanting date, recommended nitrogen rate, and potassium rate at each environment.**

| Environment | Soil Series | Soil pH | Variety | Transplant date | Base nitrogen rate | $K_2O$ |
|---|---|---|---|---|---|---|
| | | | | | ----------Kg/ha---------- | |
| BBTRS-12[1] | Goldsboro loamy sand, *Fine-loamy, siliceous, subactive, thermic AquicPaleudults* | 6.0 | NC 71 | 4/18/2012 | 74 | 87 |
| OTRS-12[2] | Helena sandy loam, *Fine, mixed, semiactive, thermic AquicHapludults* | 5.9 | CC 35 | 5/14/2012 | 80 | 148 |
| BBTRS-13[3] | Goldsboro loamy sand, *Fine-loamy, siliceous, subactive, thermic AquicPaleudults* | 5.7 | NC 196 | 4/17/2013 | 80 | 111 |
| OTRS-13[3] | Helena sandy loam, *Fine, mixed, semiactive, thermic AquicHapludults* | 6.0 | CC 27 | 5/10/2013 | 80 | 84 |
| UCPRS-13[5] | Norfolk Loamy Sand *Fine-loamy, kaolinitic, thermic TypicKandiudults* | 5.9 | NC 196 | 4/24/2013 | 80 | 94 |

[1]BBTRS-12 Whiteville, NC
[2]OTRS-12 Oxford, NC
[3]BBTRS-13 Whiteville, NC
[4]OTRS-13 Oxford, NC
[5]UCPRS-13 Rocky Mount, NC

**Table 11.Rainfall by month at each environment.**

| Month | BBTRS-12[1] | OTRS-12[2] | BBTRS-13[3] | OTRS-13[4] | UCPRS-13[5] |
|---|---|---|---|---|---|
| | ----------cm---------- | | | | |
| **April** | 5.9 | 9.9 | 5.8 | 11.6 | 6.8 |
| **May** | 22.8 | 15.6 | 57.5 | 11.6 | 7.3 |
| **June** | 5.8 | 5.7 | 65.5 | 26.3 | 25.3 |
| **July** | 20.6 | 28.1 | 31.3 | 24.1 | 12.5 |
| **August** | 36.5 | 16.5 | 16.9 | 10.7 | 11.5 |
| **September** | 10.6 | 13.8 | 12 | 4.9 | 12.3 |
| **October** | 11.4 | 6.3 | 2.7 | 5.9 | 8.3 |
| **Total** | **113.6** | **95.9** | **191.7** | **95.1** | **84** |
| **Average** | **80** | **70** | **80** | **70** | **70** |
| **Percent above average** | **41%** | **37%** | **140%** | **37%** | **20%** |

[1]BBTRS-12 Whiteville, NC; [2]OTRS-12 Oxford, NC; [3]BBTRS-13 Whiteville, NC; [4]OTRS-13 Oxford, NC; [5]UCPRS-13 Rocky Mount, NC

*State climate office of North Carolina-CRONOS database.* 2013. Retrieved February 18, 2014 from http://www.nc-climate.ncsu.edu/cronos

**Table 12.Nitrogen application timings, rates, and method.**

| Treatment Number | ----------Nitrogen Application Timing---------- | | | | | Method of Application |
|---|---|---|---|---|---|---|
| | At Transplanting | 2 Weeks after transplanting | At layby | 2 Weeks after layby | At Topping | |
| **1 (Control)** | 50% | - | 50% | - | - | NA[1] |
| **2 (Control)** | 50% | 25% | - | - | - | NA[1] |
| **3** | 25% | - | 25% | 25% | 25% | SR[2] |
| **4** | 25% | - | 25% | 25% | 25% | SA[3] |
| **5** | - | 25% | 25% | - | 50% | SR[2] |
| **6** | - | 25% | 25% | - | 50% | SA[3] |
| **7** | 50% | - | 50% | - | 25% | SR[2] |
| **8** | 50% | - | 50% | - | 25% | SA[3] |

*Treatments were the same across all environments

[1]NA, Not applicable, No "at topping" nitrogen application.
[2]SR, Stalk run-down, applied over the top of the plant,
[3]SA, Soil applied, applied to soil surface using drop lines.
*SA and SR were only for last treatment. All other treatments were applied to soil surface.

**Table 13. P values for yield, quality, value, total alkaloids, reducing sugars, leaf nitrogen content at layby, leaf nitrogen content at topping, leaf nitrogen content of cured leaf, and SPAD measurements.**

| Variable | Pr>F Env*Ntime*Nmeth | Pr>F Env*Ntime | Pr>F Env*Nmeth | Pr>F Ntime*Nmeth | Pr>F Ntime | Pr>F Nmeth |
|---|---|---|---|---|---|---|
| **Yield** | 0.1417 | 0.3806 | 0.3801 | 0.8075 | 0.2457 | 0.0456 |
| **Quality** | 0.0658 | 0.0822 | 0.2719 | 0.0008 | 0.3654 | 0.9720 |
| **Value** | 0.0344 | 0.2361 | 0.7458 | 0.0362 | 0.6047 | 0.1931 |
| **Total Alkaloids** | 0.9540 | 0.3982 | 0.9408 | 0.9242 | 0.2022 | 0.0973 |
| **Total Reducing** | 0.6334 | 0.5110 | 0.4165 | 0.7215 | 0.0439 | 0.1496 |
| **Leaf nitrogen content at layby** | 0.6924 | 0.0058 | 0.5272 | 0.1005 | 0.2924 | 0.5611 |
| **Leaf nitrogen content at topping** | 0.3820 | 0.8992 | 0.0041 | 0.4308 | 0.2077 | <.0001 |
| **Leaf nitrogen content of cured leaf** | | | | | | |
| **SPAD** | 0.8877 | 0.7962 | 0.0160 | 0.7421 | 0.2947 | 0.0629 |

Ntime, Nitrogen application timing effect
Nmeth, Nitrogen application method effect

**Table 14. Yield response to application method combined across all environments.**

| Application Method | Yield |
|---|---|
| | -----kg/ha----- |
| **Soil Applied** | 2902 a |
| **Stalk Run-down** | 2778 b |

**Table 15.Grade index response to nitrogen application rate, timing, and method combined across all environments.**

| ----------Nitrogen Application Timing---------- | | | | | Method of Application | Quality Index |
|---|---|---|---|---|---|---|
| At Transplanting | 2 Weeks after transplanting | At layby | 2 Weeks after layby | At Topping | | |
| 25% | - | 25% | 25% | 25% | SR[1] | 81 ab |
| 25% | - | 25% | 25% | 25% | SA[2] | 76 b |
| - | 25% | 25% | - | 50% | SR[1] | 74 b |
| - | 25% | 25% | - | 50% | SA[2] | 84 a |
| 50% | - | 50% | - | 25% | SR[1] | 83 a |
| 50% | - | 50% | - | 25% | SA[2] | 79 ab |

[1]SR, Stalk run-down, applied over the top of the plant.
[2]SA, Soil applied, applied to soil surface using drop lines
*SA and SR were only for last treatment. All other treatments were applied to soil surface.

**Table 16.Value response to nitrogen application rate, timing, and method at individual environments.**

| ----------Nitrogen Application Timing---------- | | | | | Method of Application | BBTRS-12[3] | OTRS-12[4] | OTRS-13[5] | UCPRS-13[6] |
|---|---|---|---|---|---|---|---|---|---|
| At Transplanting | 2 Weeks after transplanting | At layby | 2 Weeks after layby | At Topping | | ----------$/ha---------- | | | |
| 25% | - | 25% | 25% | 25% | SR[1] | 13,271 a | 12,341 a | 9,987 a | 5,881 a |
| 25% | - | 25% | 25% | 25% | SA[2] | 11,547 a | 12,956 a | 10,731 a | 5,545 ab |
| - | 25% | 25% | - | 50% | SR[1] | 10,201 a | 11,941 a | 8,925 a | 5,064 abc |
| - | 25% | 25% | - | 50% | SA[2] | 12,978 a | 12,708 a | 14,100 a | 4,461 bc |
| 50% | - | 50% | - | 25% | SR[1] | 12,829 a | 12,732 a | 13,379 a | 3,768 c |
| 50% | - | 50% | - | 25% | SA[2] | 13,911 a | 12,303 a | 10,875 a | 4,537 bc |

[1]SR, Stalk run-down, applied over the top of the plant; [2]SA, Soil applied, applied to soil surface using drop lines.
[3]BBTRS-12 Whiteville, NC
[4]OTRS-12 Oxford, NC
[5]OTRS-13 Oxford, NC
[6]UCPRS-13 Rocky Mount, NC

**Table 17. Reducing sugar percentage in response to application timing combined across all environments.**

| ----------Nitrogen Application Timing---------- | | | | | Reducing Sugars |
|---|---|---|---|---|---|
| At Transplanting | 2 Weeks after transplanting | At layby | 2 Weeks after layby | At Topping | -----%----- |
| 25% | - | 25% | 25% | 25% | 15.22 a |
| - | 25% | 25% | - | 50% | 13.66 b |
| 50% | - | 50% | - | 25% | 14.26 ab |

**Table 18.Leaf nitrogen content at layby in response to nitrogen application timing by individual environment.**

| ----------Nitrogen Application Timing---------- | | | | | Leaf Nitrogen Content at layby | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **BBTRS-12[1]** | **OTRS-12[2]** | **OTRS-13[3]** | **UCPRS-13[4]** |
| At Transplanting | 2 Weeks after transplanting | At layby | 2 Weeks after layby | At Topping | ----------%---------- | | | |
| 25% | - | 25% | 25% | 25% | 3.75 a | 4.04 a | 3.72 b | 4.11 a |
| - | 25% | 25% | - | 50% | 3.72 a | 4.78 a | 4.34 a | 3.59 b |
| 50% | - | 50% | - | 25% | 3.61 a | 4.04 a | 4.63 a | 4.15 a |

[1]BBTRS-12 Whiteville, NC
[2]OTRS-12 Oxford, NC
[3]OTRS-13 Oxford, NC
[4]UCPRS-13 Rocky Mount, NC

**Table 19.Leaf nitrogen content at topping in response to nitrogen application method.**

| Application Method | Leaf Nitrogen Content at topping | | | |
|---|---|---|---|---|
| | **BBTRS-12[1]** | **OTRS-12[2]** | **OTRS-13[3]** | **UCPRS-13[4]** |
| | ----------%---------- | | | |
| **Stalk rundown** | 2.68 a | 4.90 a | 3.63 a | 2.01 a |
| **Soil applied** | 2.50 a | 4.00 b | 3.14 b | 1.77 a |

[1]BBTRS-12 Whiteville, NC
[2]OTRS-12 Oxford, NC
[3]OTRS-13 Oxford, NC
[4]UCPRS-13 Rocky Mount, NC

**Table 20. SPAD meter readings in response to nitrogen application method.**

| Application Method | SPAD Measurements Values | | | |
|---|---|---|---|---|
| | **BBTRS-12[1]** | **OTRS-12[2]** | **OTRS-13[3]** | **UCPRS-13[4]** |
| **Stalk rundown** | 44.66 a | 46.13 a | 46.20 a | 44.60 a |
| **Soil applied** | 45.00 a | 44.28 b | 47.31 a | 38.88 b |

[1]BBTRS-12 Whiteville, NC
[2]OTRS-12 Oxford, NC
[3]OTRS-13 Oxford, NC
[4]UCPRS-13 Rocky Mount