ABSTRACT

PARKER, ROBERT GARY. Evaluation of Nitrogen Sources and Rates on Yield and Quality of Modern Flue-Cured Tobacco Cultivars. (Under the direction of Loren R. Fisher and W. David Smith.)

Nitrogen has a more pronounced effect on the growth and quality of tobacco than any other essential element even though it is not taken up in the highest quantity. Soil nitrogen regime affects plant development more than any other nutrient from seedling stage through the time of final harvest. The role of nitrogen in the development and quality of tobacco is of major importance with respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf and numerous other aspects. Soil nitrogen must be sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly insuring a quality leaf. In general, as total N in the plant increases, above the amount required for maximum growth, quality of flue-cured tobacco tends to decrease.

Field studies were conducted at two locations in 2004, 2005, and 2006 to evaluate sources and rates of nitrogen for the production of flue-cured tobacco. Calcium nitrate, ammonium nitrate, and urea ammonium nitrate (UAN) were evaluated. Sources were chosen based on previous research that provided inconsistent results when using ammonium nitrate and UAN for flue-cured production. The rates of nitrogen evaluated were 0, 22, 45, 67, and 90 kg N/ha in all years with 112 and 134 kg N/ha rates added in 2005 and 2006. All treatments received 134 kg of potassium per hectare within seven days of transplanting. Nitrogen application was applied twice with one-half of the total applied within the first week after transplanting.
and the balance applied approximately 14 days later. All other production practices followed
standard practices for the individual research stations. Nitrogen source did not affect yield,
grade index, value, total alkaloids, total reducing sugars, or leaf color. Yield increased at all
locations as nitrogen rate increased up to 67 kg/ha. Yield decreased at nitrogen rate above 90
kg/ha. Dollars per hectare followed the same trends as yield since grade index was not
affected by nitrogen rate. Total alkaloids increased as nitrogen rate increased up to 112 kg
N/ha. Inversely, total reducing sugars decreased as nitrogen rate increased. Leaf color in the
field increased as nitrogen rate increased. Soil nitrate levels increased at the highest level of
nitrogen tested at both topping and final harvest sampling times. Nitrate levels at two
intervals for treatments receiving 134 kg N/ha were almost double that of any other treatment
tested. Ammonium levels were higher, at topping, in the top 15-cm soil samples when UAN
was applied. At final harvest, soil nitrate levels were higher, in the upper 30-cm, for
treatments receiving calcium nitrate.
Evaluation of Nitrogen sources and Rates on Yield and Quality of Modern Flue-Cured Tobacco Cultivars

by
Robert Gary Parker

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Crop Science
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2009

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DEDICATION

This thesis is dedicated to my parents, Robert J. Parker, who dedicated his life to agriculture and shared that love with me and to my mother, Genelda R. Parker, who dedicated her life to her family and who taught me that hard work ‘never killed everyone’. I credit them with making me the man I am today through their love and discipline.
Robert Gary Parker (Robbie) was born in Jacksonville, North Carolina in 1973. He was raised on his family’s farm where his love for agriculture began. Robbie graduated from Swansboro High School in 1991. He attended Coastal Carolina Community College from 1992 through 1993. Robbie worked on several different farms over the next few years before continuing his education at North Carolina State University in 1999. While attending N.C. State University he worked for the USDA for two years. In the summer between his junior and senior years Robbie began to work with Dr. Alan York. After graduating with a B. S. in Agronomy in 2002, he accepted a graduate assistantship working toward a M.S. in Weed Science under Dr. Alan York. While working for Dr. York, Robbie conducted weed efficacy trials on cotton, corn, and soybean. Robbie’s graduate research focused on glyphosate-resistant corn and cotton, as well as comparing multiple glyphosate products for crop tolerance and weed efficacy. As a student, he participated in several extension field days and was a member of the NCSU Weed Team in 2002, were he his team finished first overall and he finished second place in the individual competition. In 2003 and 2004, Robbie placed first in the graduate student paper contests at both the Beltwide Cotton Conference and Southern Weed Science Society meetings and third place at the North Carolina Crop Protection School. Robbie was also named the Outstanding Master of Science Student by the Weed Science Society of North Carolina at their annual meeting in 2004. Upon completion of his M.S., Robbie was hired by Dr. David Smith as an Extension Associate to
start a pesticide residue testing program while working on a doctorate degree. While working with the tobacco group at NCSU he had the opportunity to get a lot of experience in extension working with growers to overcome greenhouse/field problems as well as doing numerous county extension and company grower meetings. Robbie worked with Dr. Smith and Dr. Loren Fisher for four years while obtaining his degree which he completed in the spring of 2008.
ACKNOWLEDGMENTS

I would like to begin by thanking my mother, Genelda R. Parker, for all of her love and support throughout my college career. She has always pushed me to be the best that I could be without forgetting where I come from. Without her behind me, I would never have been able to get through graduate school.

I wish to express my deepest appreciation to Dr. David Smith and Dr. Loren Fisher, who served as Co-chairmen of my Advisory Committee as well as mentors. I worked along side them through the tobacco extension program. I know that I will be a better person and agronomist because I have worked with the two of them over the past four years. I would also like to extend my appreciation to the other members of my committee, Dr. Michael Wagger and Dr. Randy Wells for their helpful suggestions and guidance.

Special appreciation is extended to Scott Whitley, Sherwood Wood, Zach Taylor, and Charlie Wilkinson for the help on numerous field projects during my years with the tobacco group. I would also like to acknowledge Mr. Joe Priest for all of his help in the field and for all the other day to day activities that he did to make my job easier the past four years, thank you very much.

Finally, I would like to think Dr. Bob Patterson and Dr. Alan York for being my advisors on previous degrees. Without the direction of these two men I would have not been qualified for the job that I have had for the past four years or to continue my graduate career.
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Chapter 1

Literature Review

Flue-cured tobacco (*Nicotiana tabacum* L.) is a perennial plant that is grown as an annual and has a determinate growth habit (Tso, 1990; Collins and Hawks, 1993; Peedin, 1999). Most flue-cured tobacco produced in the USA is grown on loamy sand to sandy loam soils with a low organic matter content and very little residual nitrogen from previous crops (Miner et al. 1978). These soil types allow farmers to control the amount of nitrogen available to the plant during a normal growing season. Flue-cured tobacco will begin to ripen approximately three weeks after topping and leaves will ripen from the bottom of the plant to the top. Ripening can be defined as a decrease in vigor and rate of growth, and an increase in susceptibility to shortages of water and fertilizer, specifically nitrogen (Walker, 1964). Ripening of tobacco is a naturally occurring process that takes place as nitrogen levels in the leaf decrease. Under normal production practices, two to four leaves will ripen per week and that is usually the number of leaves that will be removed per harvest (Collins and Hawks, 1993; Martin et al. 1976). This process will take place over a number of weeks and approximately four to five harvests.

The quantity of each nutrient absorbed by a field grown crop of tobacco varies considerably. Uptake is dependent on fertilization practices, residual levels of nutrients in the soil, variety and number of plants per acre, rainfall and other environmental factors (McCants and Woltz, 1967). The yield and quality characteristics of tobacco are influenced by the
balance among various physiological processes which affect leaf weight and composition or quality (Raper and McCants, 1970). Quality in the commercial sense first becomes apparent when flue-cured tobacco is taken out of the barn (Weybrew et al. 1983). However, quality is not created during curing rather it is the product of the production environment. At the moment of harvest, a leaf contains its maximum potential for quality. The environmental factor that most frequently causes alterations of leaf quality is rainfall though, more often, it is poor distribution rather than insufficiency. The association of high alkaloids and low sugars with drought tobaccos and, conversely, of low alkaloids/high sugars with wet weather crops is well known (Weybrew and Woltz, 1975; Campbell et al. 1982). The magnitude of the effects of soil moisture on quality depends on the duration and growth stage at which it occurs (McCants and Woltz, 1967). Usually the effects of insufficient or excess moisture on quality are due to excess or deficiencies in nitrogen (Elliot, 1970; Elliot and Court, 1978).

Nitrogen has a more pronounced effect on the growth and quality of tobacco than any other essential element, even though it is not taken up in the highest quantity (McCants and Woltz, 1967; Elliot and Court, 1978; Link and Terrill, 1982; Felipe and Long, 1988). From the seedling stage through the time of final harvest, the soil nitrogen regime affects plant development more than any other nutrient. With respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf and numerous other aspects, the role of nitrogen in the development and quality of tobacco is of major importance (Bowman, 1970; Elliot and Court, 1978, Weybrew et al. 1983). Soil nitrogen must be
sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly insuring a quality leaf (Elliot, 1970; Felipe and Long, 1988; Maw et al. 1995).

Grade index, which is a visual measure of the physical quality of flue-cured tobacco, has been correlated with the ratio of reducing sugars to nicotine (Gaines et al., 1983). In general, as total N in the plant increases, past the point needed to reach maturity, quality of flue-cured tobacco tends to decrease. Insufficient nitrogen produces small, thin, narrow, smooth, and pale leaves that often require harvest before they have fully ripened (Weybrew et al. 1983). The cured leaves are pale, lack texture, and the smoke is flat and insipid. Excess nitrogen produces rough, large, thin leaves. Their ripening is delayed and curing is difficult. The cured leaf color is dark and the smoke is strong and pungent (Weybrew et al. 1983; Maw et al. 1995).

McCants and Woltz (1967) stated that nitrate nitrogen is the preferred form of nitrogen for high quality tobacco, relying on results from greenhouse and field experiments. Using sand culture, Hawkins (1956) found that relative to an all nitrate solution, the growth of tobacco was reduced by one-third in solutions containing 50 percent of the nitrogen as ammonium and 80 percent when all of the nitrogen was supplied as ammonium. Skogley and McCants (1963) generated similar results from a sand culture study. In their studies, where ammonium was applied to tobacco, leaf malformations developed that were similar to those of tobacco grown on fumigated soils and fertilized with ammonium. The growth of plants fertilized with ammonium was 30% less than plants fertilized with nitrate.
Skogley and McCants also reported a reduction in uptake of other cations such as K, Ca, and Mg compared with plants fertilized with nitrate. These results follow the same pattern as other crops, such as corn, when comparing uptake of nitrate versus ammonium (Blair et al. 1970; Link and Terrill, 1982). McCants et al. (1959) had found similar results in field studies when soils were fumigated prior to ammonium application. Fumigation is believed to reduce the population of bacteria in the soil responsible for nitrification. The reduction in bacterial populations results in slower conversion of ammonium to nitrate, therefore reducing the yield and quality of tobacco. Ball-Coelho (1997) also found that growth was suppressed by high levels of ammonium fertilizer compared to equal rates of calcium nitrate when the soil was fumigated. However, in the same study UAN (urea-ammonium-nitrate) gave similar yields to that of the calcium nitrate. Ball-Coelho also found that the addition of a nitrification inhibitor further reduced nitrification when compared to fumigation alone when ammonium was used. However, when a nitrification inhibitor was used with UAN, yield and quality were similar to that of calcium nitrate alone.

Numerous field studies have been conducted which refute these findings. Under the conditions of these experiments, nitrification may occur at a rate which reduces ammonium uptake, depending on soil conditions and biological activity (McCants and Woltz, 1967). Tisdale (1952) found no differences in yield or quality among urea, nitrate, or ammonium when used as the total source of nitrogen or when used in combination with one another.
Shaw (1963) conducted field studies comparing multiple sources of nitrogen. Sodium nitrate, ammonium nitrate, and ammonium sulfate were compared as possible sources of nitrogen for production of burley tobacco. Shaw concluded that sodium nitrate, ammonium nitrate, and ammonium sulfate were equally effective as sources of nitrogen for burley tobacco when applied half at transplanting and half as side-dressing 14 days later. Williams and Miner (1982) evaluated multiple ratios of nitrate nitrogen and ammonium with and without fumigation in a three year study. Results of these studies varied from year to year based on rainfall. In years receiving higher rainfall, treatments with higher amounts of ammonium yielded up to 26% higher than treatments with only nitrate nitrogen. In years receiving less rainfall, treatments with 100% nitrate nitrogen increased yield by 6% compared to treatments which included ammonium. Yield was reduced at one location in 1978 when fumigation was included in treatments compared to non-fumigated treatments. McCants (1960) also reported lower yields when ammonium was applied to tobacco that had been fumigated prior to transplanting. Maw et al. (1995) compared a conventional granular fertilization program with 100% nitrate form of nitrogen to a liquid nitrogen program which included 32% urea ammonium nitrate. These studies found no differences in yield or quality among the treatments as long as equivalent rates of nitrogen were used. Although yield and quality were not affected, the granular program produced higher levels of alkaloids and lower levels of sugars than did the liquid treatments. Numerous other studies have been conducted evaluating nitrate to ammonium nitrogen sources for tobacco production (Grizzard et al.
Field studies compared nitrate and ammonium as individual sources of nitrogen or were combined to form various ratios of the two. In these studies no differences in yield and quality of tobacco between sources of nitrogen. As long as conditions are favorable for nitrification to take place, source of N does not affect yield and quality of tobacco (Grizzard et al. 1942; Clark et al. 1951; McCants and Woltz, 1967).

Nitrification is the conversion of ammonium to nitrate in soil and involves multiple soil microbes (Coyne, 1999). The two types of microbes which facilitate this process are chemoautotrophic nitrifiers and heterotrophic nitrifiers. The chemoautotrophic nitrification process is exclusively bacterial and is performed by a select group of bacteria. This process dominates in neutral to alkaline soils ranging from a pH of 5.7 - 10.2 and is estimated to be 100 to 1,000 times faster than heterotrophic nitrification. Soil pH levels below 5.7 have the potential to reduce levels of nitrifying bacteria which may account for the differences in results among previous tests. Historically, a soil pH of 5.0 to 5.5 was recommended for producing the best quality tobacco (McCants and Woltz, 1967; Sims and Atkinson, 1976; Cao et al., 1991). This recommendation resulted from reported increases in the incidence of black root rot (*Thielaviopsis basicola*) and black shank (*Phytophthora parasitica*) with higher pH.

Pierre (1928), conducted studies to evaluate the effect of different nitrogen fertilizers on soil pH. He found that all fertilizers containing ammonium increased soil acidity and
fertilizers which contain nitrate nitrogen increased soil pH. Pierre concluded that not all
ammonium fertilizers had the same affect on soil pH. Ammonium sulfate, which contains
two moles of NH₄, reduced the pH to a greater extent than did ammonium nitrate or urea,
which contain one mole of NH₄. The change in pH from these materials is due to the
oxidation of NH₄ to NO₃ with the production of two moles of H⁺ for each mole of NH₄
oxidized (Pierre, 1928; Reneau et al., 1968). Excess soil moisture can also affect soil microbial populations leading
to a decrease in nitrification. As water fills the soil pore space it replaces oxygen creating an
anaerobic environment. Nitrifying bacteria populations are decreased under these conditions,
reducing or stopping nitrification (Coyne, 1999).

An estimated 73% of the flue-cured tobacco produced in NC is grown in the coastal plain
region (Anonymous, 2008). This is due mainly to soil type, which is generally a sandy to
sandy loam textured soil (McCants, 1962). Since most of the tobacco soils are subject to
leaching in years of above average rainfall, nitrate application in tobacco is a potential source
of ground and surface water pollution (Miner and Sims, 1983). Nitrogen use
recommendations are based on several factors including average rainfall. Estimating
leaching losses are difficult and N leaching adjustments often are inaccurate and many times
excessive. Currently a system developed by Terry and McCants (1973) is used which
estimates losses based on an estimation of water infiltration and soil water holding capacity.
The infiltration estimate is based upon rainfall intensity, soil surface condition, plant
development and runoff. Their study indicates that sands and loamy sands will hold 1.8- to 3.0-cm of water in the top 30 cm of soil. Other studies have led to the inclusion of depth to clay layer in leaching loss recommendations (Felipe and Long, 1988; Maw et al. 1995). A number of studies have been conducted over the years examining leaf quality due to rate of nitrogen application or leaching of nitrogen leading to nitrogen deficiencies. McCants (1962) showed that when rainfall was excessive during the first nine weeks after transplanting leaching of nitrate nitrogen did occur. However, when rainfall was more uniform and rainfall events did not exceed one-half inch, leaching did not occur even when total rainfall exceeded five inches.

Different classes of tobacco (i.e. burley, flue-cured, cigar, etc.) are produced under quite different nutrient regimes (Sisson et al., 1991; Fisher et al., 2008b; Fisher et al., 2008c). Flue-cured cultivars, for example, are produced with one-fourth the nitrogen of burley cultivars. However, yields for the two types are similar (Legget et al., 1987). Crafts-Brandner et al. (1987) compared flue-cured and burley tobaccos and found that, following topping, nitrogen-use efficiency was much greater for flue-cured tobacco. Since different classes of tobacco have a different genetic basis, if can be concluded that differences among classes of tobacco, or cultivars within a class, for nitrogen-use efficiency are genetic as well. The genetic variation for nitrogen-use efficiency has been partitioned into differences in uptake and utilization of nitrogen (Pollmer et al., 1979). Moll et al. (1982) demonstrated that selection for increased nitrogen based on genetic variation was possible for corn and wheat.
Sisson et al. (1991) evaluated flue-cured cultivars for differences in nitrogen uptake and use-efficiency. Sisson evaluated 12 cultivars from three different eras in tobacco production and found that newer cultivars were more efficient at uptake and utilization of nitrogen than previously grown cultivars. Also, the more modern cultivars yielded higher than the older cultivars. He also demonstrated that modern flue-cured cultivars have more leaves which are wider and more closely spaced, when compared to older cultivars. Historically, cultivars have been chosen by breeders based on increased yield or disease resistance. Sisson stated that breeders have indirectly increased nitrogen use and uptake efficiency at the same time without testing that parameter. Sisson does not try to explain any phenotypic differences among the cultivars tested. However, his study would lead one to believe that increased root mass would also be a characteristic of the modern cultivars that would contribute to the increased nitrogen uptake. This information could be used to demonstrate that nitrogen application recommendations are too high. We could conceivably obtain the same yields using less nitrogen inputs. Sisson demonstrated that as application rate increased uptake and utilization decreased. This theory would need to be tested and it should be pointed out that the more modern cultivars tested yield from 600- to 1000 kg/ha more than the older cultivars in his study.

With the current changes in modern tobacco production, from increased soil pH, advances in current cultivars, and ever increasing fertilizer costs, I conducted research to evaluate nitrogen sources, rates, and to answer questions about the validity of our recommendations.
Should our current recommendations be based on the average yield of a specific cultivar?

Can we now use sources that contain higher amounts of ammonium nitrogen? These are a few of the questions that we hoped to answer through this research as well as trying to find a more economical approach for flue-cured fertilization.
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Chapter 2

Effect of Nitrogen Rates on Yield and Quality of NC 71 and K 346 Flue-Cured Tobacco Cultivars

ABSTRACT

Research was conducted at two locations in both 2006 and 2007 to evaluate response to nitrogen rates. Cultivars NC 71 and K 346 were selected for this study based on differences in the North Carolina Official Variety Test, with NC 71 averaging greater than 450 kg/ha more yield than K 346. Also, it has been observed that K 346 has a lower quality index than NC 71 when both varieties are produced using similar rates of nitrogen. Calcium nitrate was used as the total source of nitrogen and rates used were 45, 56, 67, 78, and 90 kg of nitrogen per hectare. Nitrogen was split applied with one half of the total applied within the first week after transplanting and the balance applied approximately 14 days later. All other production practices followed standard practices for the individual research station. There were significant main effects for variety. NC 71 averaged 3175 kg/ha while K 346 averaged 2798. There were no differences between varieties for grade index or dollars per kilogram. Dollars per hectare followed the same trend as yield with NC 71 grossing $1,247 dollars more per hectare than K 346. The main effect for nitrogen rate was also significant. Yield and dollars per hectare increased as nitrogen rate increased up to 78 kg of nitrogen per hectare. Total alkaloids increased as nitrogen increased and total reducing sugars decreased. Although yield
between the cultivars was different the trends were the same with response to nitrogen. As the nitrogen rate increased, yield for both cultivars increased with no negative affect on quality. Therefore, K 346 requires the same rate of nitrogen to achieve maximum yield as NC 71 even though there was a significant difference in yield between the two cultivars.

**INTRODUCTION**

Nitrogen has a more pronounced effect on the growth and quality of tobacco than any other essential element even though it is not absorbed in the highest quantity (McCants and Woltz, 1967; Elliot and Court, 1978; Link and Terrill, 1982; Felipe and Long, 1988). The soil nitrogen regime affects plant development more than any other nutrient from the seedling stage through the final harvest. With respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf, and numerous other aspects, the role of nitrogen in the development and quality of tobacco is of major importance (Bowman, 1970; Elliot and Court, 1978, Weybrew et al. 1983). Soil nitrogen must be sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly insuring a quality leaf (Elliot, 1970; Felipe and Long, 1988; Maw et al. 1995). Grade index, which is a visual measure of the physical quality of flue-cured tobacco, has been correlated with the ratio of reducing sugars to nicotine (Gaines et al., 1983). In general, as total N in the plant increases, quality of flue-cured tobacco tends to decrease. Insufficient nitrogen produces small, thin, smooth, and pale leaves that often require harvest before they have fully ripened
The cured leaves are pale, lack texture, and the smoke is flat and insipid. Excess nitrogen produces rough, large, thin leaves. Their ripening is delayed and curing is difficult and usually results in dark cured leaves and the smoke is strong and pungent (Weybrew et al. 1983; Maw et al. 1995).

Different classes of tobacco (i.e. burley, flue-cured, cigar, etc.) are produced under quite different nutrient regimes (Sisson et al., 1991; Fisher et al., 2008a; Fisher et al., 2008b). Flue-cured cultivars, for example, are produced with one-fourth the nitrogen of burley cultivars. However, yields for the two types are similar (Reneau et al., 1968; Raper and McCants, 1970; Legget et al., 1987). Crafts-Brandner et al. (1987) compared flue-cured and burley tobaccos and found that, following topping, nitrogen-use efficiency was much greater for flue-cured tobacco. Different classes of tobacco have a different genetic basis, giving rise to the premise that differences among classes of tobacco or varieties within a class for nitrogen-use efficiency are genetic as well. The genetic variation for nitrogen-use efficiency has been partitioned into differences in uptake and utilization of nitrogen (Pollmer et al., 1979). Moll et al. (1982) demonstrated that selection for increased nitrogen based on genetic variation was possible for corn and wheat. Sisson et al. (1991) evaluated flue-cured cultivars for differences in nitrogen uptake and use-efficiency. In this study 12 cultivars from three different eras in tobacco production were evaluated. Sisson also found that the newer cultivars were more efficient at uptake and utilization of nitrogen than previous cultivars. He also demonstrated that modern flue-cured cultivars have more leaves which are wider and more closely spaced, when compared to older cultivars.
Many of the cultivars used today for production of flue-cured tobacco have very similar pedigrees (Fisher et al. 2008b) which should produce similar yields, quality, and have similar nitrogen use efficiency rates. However, the 2005-2007 average yield for the North Carolina Official Variety Testing Program ranges from 2,870 kg/ha to 3,484 kg/ha, representing a 21% yield increase. In the OVT, all cultivars are produced with the same nitrogen rate and harvested on the same day. Yield differences may be related to differences in nitrogen use efficiency which could differ enough among varieties to account for the variation in yield. The grade index is also lower for the lower yielding varieties.

Over-fertilization of tobacco harvest may lead to a low grade index because of green or variegated cured leaf color. All varieties tested are planted, fertilized and harvested in the same manner, which may contribute to this problem. Therefore, the objective of this experiment was to evaluate the response of a high and low yielding cultivar to nitrogen rate.

**MATERIALS AND METHODS**

Three experiments were conducted at two locations in North Carolina from 2006-2007. Locations were the Upper Coastal Plain Research Station near Rocky Mount, NC, and the Border Belt Tobacco Research Station near Whiteville, NC, in 2006 and 2007. Soil types in Rocky Mount were a Norfolk sandy loam (fine loamy, siliceous, thermic Typic Paleudult) with a pH of 5.9 in 2006 and an Aycock very fine sandy loam (fine silty, siliceous, thermic Typic Paleudult) with a pH of 6.0 in 2007. Soil type in Whiteville was a Lynchburg fine
sandy loam (fine loamy, siliceous, thermic Aeric Paleaquult) with a pH of 6.0.

Trials were conducted using a factorial arrangement of treatments with factors being variety and nitrogen rate. The varieties were NC 71 and K 346, and nitrogen rates were 45, 56, 67, 78, 90 kg/ha. Treatments were arranged in a split-block using a randomized complete block design with four replications. Varieties were the whole block factor, the nitrogen rates were randomized within the block. Each experimental plot consisted of four rows containing 22 plants. In-row plant spacing was 56 cm and row spacing was 122 cm wide by 13.5 m long. Data were collected from the center two rows of the four row plot.

Flue-cured tobacco transplants were produced and transplanted in accordance with normal production practices by the respective research stations. Cultivars were transplanted at Whiteville the last week in April and were transplanted in Rocky Mount the first week in May in all years. Plant populations were 14,800 per hectare at all locations. Potassium magnesium sulfate (0-0-22) was applied in a band 15 cm to the side and 10 cm deep at a rate of 134 kg/ha 5- to 7-days after transplanting. One-half of the nitrogen was applied by hand on the same day as the potassium application. The balance of the nitrogen was applied approximately two weeks after the initial nitrogen application. Calcium nitrate was applied as the nitrogen source for all treatments. No phosphorous was added as all locations had phosphorous levels greater than 240 kg/ha according to soil test reports provided by the research station (data not shown). All other cultural practices, control of axillary buds, harvesting, and curing procedures were consistent with North Carolina Agricultural Extension Service recommendations (Fisher et al., 2008c).
Rainfall distribution during each of the two growing seasons was variable, and the crops were irrigated as needed to provide a uniform moisture regime. Leaching was not considered a factor during either of the two years.

Cured leaf samples from four harvests were composited on a weighted-mean basis by harvest position from each plot for chemical analysis. Lamina was removed from the mid-vein, dried, and ground to pass through a 1-mm sieve. Nicotine and reducing sugars were determined using methods developed by Harvey et al. (1969). Total cured leaf yield was obtained for each plot. An Official Standard Grade (Fisher et al., 2008b) was assigned to each priming, and a grade index indicating physical leaf quality, was determined for each plot. Value per hectare and value per kilogram were computed for each plot using a standard price index for each grade.

Data were subjected to ANOVA with partitioning appropriate for the factorial treatment arrangements. In the analysis, years and locations were assumed random, and nitrogen rates and varieties were considered fixed.

**RESULTS AND DISCUSSION**

The were no year or location interactions observed for any of the treatments in 2006 or 2007. Therefore, all data were pooled over years and locations. There were no interactions or main effects for dollars per kilogram or quality index (Table 1).

There was not a cultivar by nitrogen rate interaction and the yield for each cultivar followed the same trend with yield increasing as nitrogen rate increased reaching a plateau at
78 kg/ha (Figure 1). However, there was a main effect for nitrogen rate (Table 2). Yield increased as nitrogen rate increased up to 78 kg/ha. No yield increase was observed above 78 kg/ha. Dollars per hectare increased as nitrogen rate increased up to 78 kg/ha. Dollars per hectare followed the same trend as yield as quality index was similar for all rates of nitrogen. Total alkaloids increased as nitrogen rate increased. Ninety kg/ha produced 3.29 percent alkaloids which was significantly higher than all other treatments. Inversely, reducing sugars decreased as nitrogen rate increased. Forty-five kg/ha nitrogen produced the highest total reducing sugars at 15.4 percent which was not different than 56 or 67 kg/ha nitrogen. An increase in total alkaloids and decrease in reducing sugars as nitrogen rate increases follows results previously reported by (McCants and Woltz, 1967; Elliot, 1970; Weybrew and Woltz, 1975; Link and Terrill, 1982; Court and Hendel, 1986 Flowers, 1999).

There was a main effect for cultivar (Table 3). Variety NC 71 yield was significantly higher than K 346 with NC 71 producing 3175 kg of tobacco per hectare compared to K 346 which produced 2798 kg/ha. Variety K 346 produced approximately 12 percent less yield which followed data presented from the three year OVT (Fisher et al., 2008b). Quality index and dollars per kg were similar for both varieties. Dollars per hectare were significantly different with NC 71 averaging $1,247 per hectare more than K 346. Total alkaloids were similar for both varieties with NC 71 and K 346 averaging 2.92 and 2.88 percent, respectively. NC 71 had significantly higher total reducing sugars than K 346. Total reducing sugars were 14.4 and 13.03 percent for NC 71 and K346, respectively. Since there were no differences observed between the two varieties for total alkaloids and with NC 71
producing higher yields the fact that NC 71 produced higher amounts of carbohydrates that reduced to sugars would be expected.

**SUMMARY**

Variety NC 71 produced higher yields and return per hectare than did K 346 (Table 2). Yield and return per hectare increased as nitrogen rate increased up to 78 kg/ha (Table 1). Total alkaloids were similar with both varieties, however, total alkaloids increased as nitrogen rate increased. Grade index was similar for both varieties at all rates of nitrogen applied. We did not see an increase in the grade index for K 346 when lower rates of nitrogen were applied. Therefore we can not conclude with certainty that nitrogen rates should be reduced for lower yielding varieties such as K 346. NC 71 did produce higher total reducing sugars, however, this can be attributed to the higher overall yield and similar percent total alkaloids (Table 2).

Since there was not a variety by nitrogen rate interaction, nitrogen recommendations to growers should be the same for both varieties. K 346 is a variety that has good disease resistance for race 0 and 1 black shank (*Phytophthora parasitica var. nicotianae*) and Granville wilt (*Ralstonia solanacearum*) (Mila and Radcliff, 2008). All cultivars on the market that are equal to or higher in disease resistance than K 346 yield similarly to K 346 (Fisher et al., 2008). The genetic makeup of K 346, and possibly other cultivars highly resistant to race 1 black shank and Granville wilt, could directly affect the nitrogen uptake and utilization characteristics of these cultivars reducing the yield compared to cultivars such
as NC 71.
LITERATURE CITED


Table 1. Analysis of variance (p-value).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Yield</th>
<th>Index&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Price&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Alkaloids</th>
<th>Reducing Sugars</th>
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<tr>
<td>Rep(exp)</td>
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<td>0.0904</td>
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<td>0.0001</td>
</tr>
<tr>
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<td>0.3523</td>
<td>0.0026</td>
<td>0.4344</td>
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<td>0.0113</td>
</tr>
<tr>
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<td>0.4103</td>
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</tr>
<tr>
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<td>0.4940</td>
<td>0.8893</td>
<td>0.6775</td>
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</tbody>
</table>

<sup>a</sup> Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

<sup>b</sup> Value = Gross return per hectare (yield x price).

<sup>c</sup> Price = Dollars per kilogram of tobacco.
Table 2. Effect of nitrogen rate on yield quality and value averaged over years, locations, and cultivars.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Rate kg/ha</th>
<th>Grade Index\textsuperscript{b}</th>
<th>Value\textsuperscript{c} $/ha</th>
<th>Price\textsuperscript{d} $/kg</th>
<th>Alkaloids</th>
<th>Reducing Sugar %</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>79 a</td>
<td>8030 d</td>
<td>2.82 a</td>
<td>2.52 d</td>
<td>15.4 a</td>
</tr>
<tr>
<td>56</td>
<td>81 a</td>
<td>8349 cd</td>
<td>2.91 a</td>
<td>2.67 d</td>
<td>14.5 a</td>
</tr>
<tr>
<td>67</td>
<td>80 a</td>
<td>8741 bc</td>
<td>2.89 a</td>
<td>2.88 c</td>
<td>14.6 a</td>
</tr>
<tr>
<td>78</td>
<td>81 a</td>
<td>9522 a</td>
<td>2.95 a</td>
<td>3.13 b</td>
<td>12.4 b</td>
</tr>
<tr>
<td>90</td>
<td>82 a</td>
<td>9146 ab</td>
<td>2.98 a</td>
<td>3.29 a</td>
<td>11.7 b</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>0.0004</td>
<td>0.09</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\textsuperscript{b} Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

\textsuperscript{c} Value = Gross return per hectare (yield x price).

\textsuperscript{d} Price = Dollars per kilogram of tobacco.
Table 3. Effect of cultivars on yield, quality, and value averaged over years, locations, and varieties.\(^a\)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Yield (^b) kg/ha</th>
<th>Index (^b) 1-100</th>
<th>Value (^c) $/ha</th>
<th>Price (^d) $/kg</th>
<th>Alkaloids</th>
<th>Reducing Sugar %</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 346</td>
<td>2798 b</td>
<td>81 a</td>
<td>8134 b</td>
<td>2.91 a</td>
<td>2.88 a</td>
<td>13.03 b</td>
</tr>
<tr>
<td>NC 71</td>
<td>3175 a</td>
<td>80 a</td>
<td>9381 a</td>
<td>2.89 a</td>
<td>2.92 a</td>
<td>14.40 a</td>
</tr>
<tr>
<td>LSD</td>
<td>137</td>
<td>1.71</td>
<td>422</td>
<td>1.3</td>
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</tr>
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<td>p-value</td>
<td>0.0028</td>
<td>0.3523</td>
<td>0.0026</td>
<td>0.4344</td>
<td>0.6293</td>
<td>0.0113</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at \(P = 0.05\).

\(^b\) Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

\(^c\) Value = Gross return per hectare (yield x price).

\(^d\) Price = Dollars per kilogram of tobacco.
Fig. 1. Relationship between nitrogen rate and cured leaf yield in 2006 and 2007.

\[ y = 1608.42 + 35.14x - 0.20744x^2 \]

\[ (r^2 = 0.76, P = 0.0232) \]
Chapter 3

Nitrogen Release Characteristics of Urea Treated with Agrotain¹ on
Yield and Quality of Flue-Cured Tobacco

ABSTRACT

Research was conducted at one location in 2006 and two locations in 2007 in order to
determine if fertilization with urea or urea plus Agrotain (urease inhibitor) would produce
flue-cured tobacco similar to that obtained from a nitrogen regime of all nitrate nitrogen.
Treatments consisted of three sources of nitrogen: calcium nitrate, urea, and urea plus
Agrotain and applied at rates to provide 56 and 78 kg of nitrogen per hectare. Nitrogen
source did not affect yield, grade index, dollars per kilogram or total reducing sugars.
However, nitrogen source did affect total value and total alkaloids. Increasing the nitrogen
rate from 56 to 78 kg/ha increased dollars per hectare by $417 and increased total alkaloids
from 3.46 to 3.67 percent. Calcium nitrate produced a higher return per hectare than did urea
or urea plus Agrotain. Rainfall was normal for 2006 but was lower than normal for 2007,
which could have slowed the rate of nitrification. From these experiments we conclude that
urea or urea plus Agrotain will not produce flue-cured tobacco of equal value or similar
alkaloid levels as an all nitrate regime under the environmental conditions.

¹ Agrotain, NBPT (N-(n-butl) thiophosphoric triamide), AGROTAIN International LLC,
Saint Louis, MO.
INTRODUCTION

Tobacco is a very important to the economy of North Carolina and remains the highest revenue crop produced in the state. In 2007, tobacco accounted for more the 7% (575 million dollars) of the total agricultural receipts in North Carolina, and more than 28% of the crop receipts (Anonymous, 2008).

Most of the flue-cured tobacco (Nicotiana tabacum) in the United States is grown on sandy loam or loamy sand textured soils. These soils are low in organic matter, have low cation exchange and water holding capacities, and frequently are subject to leaching of plant nutrients by excessive rainfall (Miner et al., 1978). Flue-cured tobacco requires precise levels of nitrogen in order to achieve maximum yield and quality. The amount of nitrogen supplied can be controlled through fertilization. The nitrogen fertilization program affects plant development more than any other nutrient from the seedling stage through the final harvest. With respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf and numerous other aspects, the role of nitrogen in the development and quality of tobacco is of major importance (Raper and McCants, 1966; Raper and McCants, 1967; Bowman, 1970; Elliot and Court, 1978, Weybrew et al. 1983). Soil nitrogen must be sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly insuring a quality leaf (Elliot, 1970; Weybrew et al., 1983; Felipe and Long, 1988; Maw et al. 1995).

Historically, fertilizer recommendations for flue-cured tobacco have recommended at least
50% of the nitrogen be applied in the form of nitrate (Skogley and McCants, 1963; McCants and Woltz, 1967; Elliot, 1970; Miner and Sims, 1983; Collins and Hawks, 1993) to maximize yield and quality. This recommendation is due to studies conducted which found that tobacco grown in soil with high concentrations of ammonium reduced yield 30 to 80 percent compared to tobacco produced using at least 50 percent nitrate (Hawkins, 1956; McCants et al., 1959; Skogley and McCants, 1963; Ball-Coelho, 1997). However, these studies were conducted in greenhouses under conditions with slow to no nitrification. Also, when field studies were conducted in this time period soil pH was maintained from 5.0 to 5.5 in order to reduce disease (McCants and Woltz, 1967; Sims and Atkinson, 1976). Low soil pH reduces soil nitrifying bacteria populations thereby reducing the rate of nitrification (Coyne, 1999).

Very little research has been conducted evaluating urea as a source of nitrogen in flue-cured tobacco production. Miner et al., (1978) evaluated isobutylidene diurea (IBDU) as a source of nitrogen because it is less leachable than a nitrate source. Isobutylidene diurea did reduce leaching and under simulated leaching field studies produced higher yields than did sodium nitrate. However, under normal growing conditions sodium nitrate produced higher yields than did IBDU. Williams and Miner (1982), evaluated urea as a source of nitrogen for tobacco production. The multi-year study produced results showing that in a high rainfall year the treatments receiving urea out yielded sodium nitrate by as much as 26 percent. However, in a low rainfall year sodium nitrate produced 7 percent more yield. Williams concluded that under conditions of adequate and well-distributed rainfall, both sources of
nitrogen had similar effects on yield and quality.

From 2002 to 2008 fertilizer costs for products typically used in the production of flue-cured tobacco have more than doubled (Bullen and Brown, 2002; Brown 2008). This increase has led growers to examine alternative sources of nitrogen to reduce input costs. Therefore, the object of this study was to evaluate urea and urea plus a urease inhibitor on yield and value of flue-cured tobacco. Also, to determine if urea plus the urease inhibitor decreased uptake of nitrogen in the nitrate form and led to a reduction in yield or quality.

MATERIALS AND METHODS

Three tobacco experiments were conducted at two locations in North Carolina from 2006-2007. Locations were the Upper Coastal Plain Research Station near Rocky Mount, NC, in 2006 and 2007, and at the Border Belt Tobacco Research Station near Whiteville, NC, in 2007. Soil types in Rocky Mount were a Norfolk sandy loam (fine loamy, siliceous, thermic Typic Paleudult) with a pH of 5.9 in 2006 and an Aycock very fine sandy loam (fine silty, siliceous, thermic Typic Paleudult) with a pH of 6.0 in 2007. Soil type in Whiteville was a Lynchburg fine sandy loam (fine loamy, siliceous, thermic Aeric Paleaquult) with a pH of 6.0.

The factorial arrangement of treatments consisted of three sources of nitrogen (calcium nitrate, urea, and urea plus a urease inhibitor) and two rates of nitrogen fertilization (56 and 78 kg/ha). Treatments were arranged in a randomized complete block design. Each experimental plot consisted of four rows each containing 22 plants. Plant spacing within the row was 56 cm and 122 cm between rows. Data were collected from the center two rows of
a four row plot. Treatments at all locations were replicated four times.

Flue-cured tobacco transplants were produced and transplanted in accordance with normal production practices by the respective research stations. Variety NC 71 was transplanted at Whiteville the last week in April and NC 71 was transplanted in Rocky Mount the first week in May in both years. Plant populations were 14,800 per hectare at all locations. Potassium magnesium sulfate (0-0-22) was applied in a band 15 cm to the side and 10 cm deep at a rate of 134 kg/ha 5- to 7-days after transplanting. One-half of the nitrogen was applied by hand on the same day as the potassium application. The balance of the nitrogen was applied approximately two weeks after the initial nitrogen application. Fertilizer phosphorous was not applied because all locations had phosphorous levels greater than 240 kg/ha according to soil test analysis provided by the Research Station (data not shown). All other cultural practices, control of axillary buds, harvesting, and curing procedures were consistent with North Carolina Cooperative Extension Service recommendations.

Rainfall distribution during each of the two growing seasons was variable, and the crops were irrigated as needed. Leaching was not considered a factor either year.

Cured leaf samples from four harvests were composited on a weighted-mean basis by primings from each plot for chemical analysis. Lamina was removed from the mid-vein, dried, and ground to pass through a 1-mm sieve. Nicotine and reducing sugars were determined using methods developed by Harvey et al. (1969). Total cured leaf yield was obtained for each plot. An Official Standard Grade was assigned to each priming and a grade index indicating physical leaf quality, was determined for each plot (Fisher et al., 2008).
Value per hectare and value per kilogram were computed for each plot using a standard price index for each grade.

Data were subjected to ANOVA with partitioning appropriate for the factorial treatment arrangements. In the analysis, years and locations were assumed random, and nitrogen rates and varieties were considered fixed.

**RESULTS AND DISCUSSION**

There were no year or location interactions or main effects (Table 1), therefore all data were averaged over years and locations. There were no nitrogen rate interactions or main effects for yield, quality index, dollars per kilogram or total reducing sugars at the $p \leq 0.05$.

Application of 78 kg/ha nitrogen increased crop value as measured by $$/ha (Table 2). Yield was not significant at the $p \leq 0.05$, however, it was significant at the $p \leq 0.1$ level. At the $p \leq 0.1$ level yield increased as nitrogen rate increased. Yield increased from 2914 kg/ha to 3039 kg/ha as the nitrogen rate increased from 56 to 78 kg/ha. The trend for increased yield and similar grade index led to the difference in dollars per hectare.

There was a nitrogen rate main effect for total alkaloids (Table 2). Total alkaloids increased as nitrogen rate increased. An increase in total alkaloids as nitrogen rate increases follows results previously reported by: McCants and Woltz, 1967; Elliot, 1970; Weybrew and Woltz, 1975; Link and Terrill, 1982; Court and Hendel, 1986 Flowers, 1999.

There were no nitrogen source interactions or main effects for yield, quality index, dollars per kilogram, total alkaloids, or total reducing sugars (Table 1). There was, however, a
nitrogen source main effect for dollars per hectare (Table 3). Calcium nitrate had a higher value per hectare than did treatments receiving urea or urea plus Agrotain. Dollars per hectare were $9,258, $8840, and $8818 for calcium nitrate, urea, and urea plus Agrotain, respectively. These differences can be attributed to the numerical differences among sources for yield and dollars per kilogram, that when multiplied together to give us our dollars per hectare.

SUMMARY

No differences were found between nitrogen sources or rates for yield, quality index, dollars per kilogram or total reducing sugars (Table 1). Dollars per hectare were significant for both nitrogen sources and rate. Increasing the nitrogen rate from 56 to 78 kg/ha increased dollars per hectare by $417 and increased total alkaloids from 3.46 to 3.67 percent (Table 2). Calcium nitrate produced a higher return per hectare than did urea or urea plus Agrotain (Table 3). These results agree with previous results published by McCants and Woltz, (1967). Williams and Miner (1982), reported similar results with sodium nitrate producing greater yield and similar dollars per acre than urea in a dry season. However, they found that in wet seasons urea produced up to 26 percent higher yields due to urea being less leachable than nitrate nitrogen. The locations for these tests in 2006 experienced normal rainfall for each location. However, locations in 2007 were extremely dry and could have led to slower nitrification rates.

Urea is not currently recommended as a sole source of nitrogen for flue-cured tobacco
data from this study indicate urea alone is not as good as a nitrate alone source for the production of flue-cured tobacco. However, in this study all plots were harvested at the same time regardless of nitrogen rate or source due to time constraints at the research station. This could have biased the data toward the nitrate source which would have matured sooner than the urea treated plots. Plots treated with urea generally appeared greener than nitrate treated plots, specifically those treated with the urease inhibitor. This could have lead to slightly lower grade index than the nitrate treated plots.

This study agrees with many other studies looking at urea as a nitrogen source for flue-cured tobacco. Williams and Miner (1982) found that in dry years a nitrate source of nitrogen out yielded urea applied alone. However, Miner (1978) demonstrated that sources of slow release urea could be used in flue-cured production and would produce increased yields compared to nitrate sources under leaching conditions. The results of this study show issues with the research methods. By harvesting all plots at the same time we may have influenced the results. If you look at yield, grade index or price, there are no differences and this would lead to the conclusion that there are no differences. However, value (yield X price) is significant which points to a nitrate source producing a higher value under the conditions of this experiment.
LITERATURE CITED


Table 1. Analysis of variance (p-value).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Yield</th>
<th>Index&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Price&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Alkaloids</th>
<th>Sugars</th>
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<tr>
<td>Exp</td>
<td>2</td>
<td>0.2615</td>
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<td>0.0841</td>
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<sup>a</sup> Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

<sup>b</sup> Value = Gross return per hectare (yield x price).

<sup>c</sup> Price = Dollars per kilogram of tobacco.
Table 2. Effect of nitrogen rate on yield, grade index, value, price, total alkaloids, and reducing sugars averaged over years, locations, and sources.a

<table>
<thead>
<tr>
<th>Rate</th>
<th>Yield</th>
<th>Grade Index</th>
<th>Value</th>
<th>Price</th>
<th>Total Alkaloids</th>
<th>Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg/ha</td>
<td>kg/ha</td>
<td>$/ha</td>
<td>$/kg</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>56</td>
<td>2914 a</td>
<td>80 a</td>
<td>8764 b</td>
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<td>3.46 b</td>
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<tr>
<td>78</td>
<td>3039 a</td>
<td>81 a</td>
<td>9181 a</td>
<td>3.00 a</td>
<td>3.67 a</td>
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</tr>
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<td>0.7071</td>
<td>0.0312</td>
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</table>

a Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05. Nitrogen sources: Calcium nitrate, urea, and urea plus urease inhibitor.

b Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

c Value = Gross return per hectare (yield x price).

d Price = Dollars per kilogram of tobacco.
Table 3. Effect of nitrogen source on yield, grade index, value, price, total alkaloids, and reducing sugars averaged over years, locations, and nitrogen rates.a

<table>
<thead>
<tr>
<th>Source</th>
<th>Yield</th>
<th>Grade Index b</th>
<th>Value c</th>
<th>Price d</th>
<th>Total Alkaloids</th>
<th>Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/ha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaNO₃</td>
<td>3041 a</td>
<td>81 a</td>
<td>9258 a</td>
<td>3.02 a</td>
<td>3.69 a</td>
<td>11.70 a</td>
</tr>
<tr>
<td>Urea</td>
<td>2919 a</td>
<td>81 a</td>
<td>8840 b</td>
<td>3.00 a</td>
<td>3.52 a</td>
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<td>Urea + Inhibitor</td>
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<td>8818 b</td>
<td>2.95 a</td>
<td>3.46 a</td>
<td>12.44 a</td>
</tr>
<tr>
<td>LSD</td>
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<td>152</td>
<td>0.15</td>
<td>0.56</td>
<td>2.08</td>
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<td>0.5247</td>
<td>0.6441</td>
<td>0.7133</td>
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</tbody>
</table>

a Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

b Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

c Value = Gross return per hectare (yield x price).

d Price = Dollars per kilogram of tobacco.
Chapter 4

Evaluation of Nitrogen Sources and Rates on Yield and Quality of Flue-Cured Tobacco

ABSTRACT

Research was conducted at two locations in 2004, 2005, and 2006 to evaluate sources and rates of nitrogen for the production of flue-cured tobacco. Calcium nitrate, ammonium nitrate, and urea ammonium nitrate (UAN) were evaluated. Sources were chosen based on previous research that provided inconsistent results when using ammonium nitrate and UAN for flue-cured production. The rates of nitrogen evaluated were 0, 22, 45, 67, and 90 kg N/ha in all years with 112 and 134 kg N/ha rates added in 2005 and 2006. All treatments received 134 kg of potassium per hectare within seven days of transplanting. Nitrogen application was applied twice with one-half of the total applied within the first week after transplanting and the balance applied approximately 14 days later. All other production practices followed standard practices for the individual research stations. Nitrogen source did not affect yield, grade index, value, total alkaloids, total reducing sugars, or leaf color. Yield increased at all locations as nitrogen rate increased up to 67 kg/ha. Yield decreased at nitrogen rate above 90 kg/ha. Dollars per hectare followed the same trends as yield since grade index was not affected by nitrogen rate. Total alkaloids increased as nitrogen rate increased up to 112 kg N/ha. Inversely, total reducing sugars decreased as nitrogen rate increased. Leaf color in the field increased as nitrogen rate increased. Soil nitrate levels increased at the highest level of
nitrogen tested at both topping and final harvest sampling times. Nitrate levels at two intervals for treatments receiving 134 kg N/ha were almost double that of any other treatment tested. Ammonium levels were higher, at topping, in the top 15-cm of soil when UAN was applied. At final harvest, soil nitrate levels were higher in the upper 30-cm for treatments receiving calcium nitrate.

INTRODUCTION

Nitrogen has a more pronounced effect on the growth and quality of tobacco than any other essential element even though it is not taken up in the highest quantity (McCants and Woltz, 1967; Elliot and Court, 1978; Link and Terrill, 1982; Felipe and Long, 1988). Soil nitrogen regime affects plant development more than any other nutrient from seedling stage through the time of final harvest. The role of nitrogen in the development and quality of tobacco is of major importance with respect to time of absorption, form of nitrogen absorbed, rate of application, concentration in the leaf and numerous other aspects, (Bowman, 1970; Elliot and Court, 1978, Weybrew et al. 1983). Soil nitrogen must be sufficient during early and mid-season growth stages to ensure vigorous, but not excessive growth, and it should be nearly depleted by flowering for the plant to mature and ripen properly insuring a quality leaf (Elliot, 1970; Felipe and Long, 1988; Maw et al. 1995). In general, as total N in the plant increases, quality of flue-cured tobacco tends to decrease (Weybrew et al. 1983). Grade index, which is a visual measure of the physical quality of flue-cured tobacco, has been correlated with the ratio of reducing sugars to nicotine (Gaines et al., 1983).
Based on field and greenhouse studies, McCants and Woltz (1967) stated that nitrate nitrogen is the preferred form of nitrogen for high quality tobacco. Hawkins (1956), using sand culture, found that relative to an all nitrate solution, the growth of tobacco was reduced one-third in solutions containing 50 percent of the nitrogen as ammonium and 80 percent when all of the nitrogen was supplied as ammonium. Skogley and McCants (1963) generated similar results from a sand culture study. In their studies, where ammonium was applied to tobacco, it caused the development of leaf malformations similar to those of tobacco grown on fumigated soils and fertilized with ammonium. The growth of plants fertilized with ammonium was 30% less than plants fertilized with nitrate. Skogley and McCants also reported a reduction in uptake of other cations such as K, Ca, and Mg compared with plants fertilized with nitrate. These results follow the same pattern as other crops, such as corn, when comparing uptake of nitrate versus ammonium (Blair et al. 1970; Link and Terrill, 1982). Ball-Coelho (1997) also found that growth was suppressed by high levels of ammonium fertilizer compared to equal rates of calcium nitrate when the soil was fumigated. However, in the same study UAN (urea-ammonium-nitrate) gave similar yields to that of the calcium nitrate.

Numerous field studies have been conducted which refute these findings. Under the conditions of these experiments, nitrification may occur at a rate which reduces ammonium uptake, depending on soil conditions and biological activity (McCants and Woltz, 1967). Tisdale (1952) found no differences in yield or quality among urea, nitrate, or ammonium when used as the total source of nitrogen or when used in combination with one another.
Shaw (1963) conducted field studies comparing multiple sources of nitrogen. Sodium nitrate, ammonium nitrate, and ammonium sulfate were compared as possible sources of nitrogen for production of burley tobacco. Shaw concluded that sodium nitrate, ammonium nitrate, and ammonium sulfate were equally effective as sources of nitrogen for burley tobacco when applied half at transplanting and half as side-dressing 14 days later. Williams and Miner (1982) evaluated multiple ratios of nitrate nitrogen and ammonium from urea with and without fumigation in a three year study. Results of these studies varied based on rainfall. In years receiving higher than normal rainfall, treatments with higher amounts of ammonium yielded up to 26% higher than treatments with only nitrate nitrogen. In years receiving less rainfall treatments with 100% nitrate nitrogen increased yield by 6% compared to treatments which included ammonium. Yield was reduced at one location in fumigated soils compared to non-fumigated soils. Maw et al. (1995) compared a conventional granular fertilization program with 100% nitrate nitrogen to a liquid nitrogen program which included 32% urea ammonium nitrate which provides 7.5% nitrate nitrogen. These studies found no differences in yield or quality among the treatments as long as equivalent rates of nitrogen were used. Numerous other studies have been conducted comparing nitrate nitrogen to ammonium as sources for tobacco production (Grizzard et al. 1942; Tisdale, 1952; Shaw, 1957; Peterson, 1964; Elliot, 1970; Court and Hendel, 1986). Studies compared nitrate and ammonium both individually or combined to form various ratios of the two. These studies found no differences between sources of nitrogen for yield or quality of tobacco. The authors concluded that nitrification of ammonium takes place rapidly under optimum field
conditions thereby preventing toxic amounts of ammonium from being absorbed by tobacco plants (Grizzard et al. 1942; Clark et al. 1951; McCants and Woltz, 1967).

Research has shown that more modern cultivars of flue-cured tobacco are more efficient at uptake and utilization of nitrogen. Sisson et al. (1991) evaluated flue-cured cultivars for differences in nitrogen uptake and use-efficiency. Twelve cultivars from three different eras in tobacco production were evaluated. Sisson found that modern cultivars were more efficient at uptake and utilization of nitrogen than previously grown cultivars. He also demonstrated that modern flue-cured cultivars have more leaves which are wider and more closely spaced, when compared to older cultivars, which may be the reason for their increased yield. However, it is not known whether current N recommendations are accurate given the changes in N uptake and utilization with modern cultivars (Fisher et al., 2008)

Even though recent research supports the use of ammonium based nitrogen programs, growers have been reluctant to change fertilization programs. Until recently, fertilizers have not been a significant cost of production and alternative fertilizer programs have not resulted in a significant savings for the grower (Brown, 2002). However, in the past two years the cost of fertilizer has more than doubled (Bullen and Brown, 2008) forcing growers to look at cheaper sources of nitrogen. Therefore, the objectives of this research were to evaluate sources of nitrogen on the yield and quality of flue-cured tobacco and to evaluate the effects of current nitrogen sources and rate on yield and quality.
MATERIALS AND METHODS

Six tobacco experiments were conducted at two locations in North Carolina from 2004 to 2006. Locations were the Lower Coastal Plain Research Station near Kinston, NC, and at the Oxford Tobacco Research Station near Oxford, NC, each year. Soil type in Kinston was a Norfolk sandy loam (fine loamy, siliceous, thermic Typic Paleudult) with a pH of 6.0 in 2004, 5.5 in 2005, and 6.4 in 2006. Soil types in Oxford were a Vance sandy loam (clayey, mixed, thermic Typic Hapludult) with a pH of 6.0 in 2004, and a Helena loamy sand (clayey, mixed, thermic Aquic Hapludult) with a pH of 5.9 in 2005 and a pH of 6.1 in 2006.

Treatments consisted of a factorial arrangement of three sources of nitrogen (calcium nitrate, ammonium nitrate, and 32% urea ammonium nitrate (UAN)) and five rates of nitrogen fertilization (0, 22, 45, 67 and 90 kg/ha) in 2004 and seven rates (0, 22, 45, 67, 90, 112, and 134 kg/ha) in 2005 and 2006. Treatments were arranged in a randomized complete block design. Each experimental plot consisted of four rows containing 22 plants. In row plant spacing was 56 cm and row spacing was 122 cm wide by 13.5 m long. Data were collected from the center two rows of the four row plots. Treatments at all locations were replicated four times.

Flue-cured tobacco transplants were produced and transplanted in accordance with normal production practices by the respective research stations. Variety NC 71 was transplanted at Kinston the last week in April in all years and NC 297 was transplanted in Oxford the second week in May in all years. Plant populations were 14,800 per hectare at all locations. A potassium blend of potassium magnesium sulfate (0-0-22), sulfate of potash (0-0-50) and
murate of potash (0-0-60) was used for the potassium source and was applied in a band 15 cm to the side and 10 cm deep at a rate of 134 kg/ha 5- to 7-days after transplanting. One-half of the nitrogen was applied on the same day as the potassium application. The dry sources of nitrogen were pre-weighed for each plot and applied by hand in the same band as the potassium blend. The 32% UAN was applied with a CO2-pressurized backpack sprayer equipped with one flood nozzle calibrated to deliver the appropriate volume for each rate. The balance of the nitrogen was applied with the same methods, previously described, approximately two weeks after the initial nitrogen application. Phosphorous was not applied because all locations had a phosphorous level greater than 240 kg/ha according to soil test reports provided by the Research Station (data not shown). Tests at the Kinston location were treated with Telon C-17 at 117 L/HA (dichlorpropene + chloropicrin) in all years. All other cultural practices were consistent with North Carolina Cooperative Extension Service (Fisher et al. 2008b).

Rainfall distribution during each of the three growing seasons was variable, and the crops were irrigated as needed to provide a uniform moisture regime. Leaching was not considered a factor during any of the three years.

Relative chlorophyll measurements were made at the button stage of plant growth using the Minolta chlorophyll meter SPAD-502\(^2\). The SPAD-502 is a portable, non-destructive meter which measures red region transmittance peak of chlorophyll a and b (around 660 nm) of the leaf and calculates a numerical SPAD value which is proportional to the amount of

\(^2\) Minolta Camera Co., Ltd. 3-13, 2-Chrome, Azuchi-Machi, Chuo-Ku, Osaka 541 Japan.
chlorophyll present in the leaf (Anonymous, 1989). Leaf greenness is indicative of leaf chlorophyll content, therefore SPAD values can quantify visual subjectivity of leaf color. Higher SPAD values correspond to a greener leaf.

Soil samples were taken on selected treatments three times during the growing season for all tests to determine the concentration of residual NO₃-N and NH₄-N. Samples were taken prior to the application of any nitrogen, just after removal of the flower (mid-season), and within seven days of the final harvest (late-season). The first set of samples were taken from random plots in order to obtain the relative levels of nitrate and ammonium in the soil prior to application of nitrogen treatments. Subsequent samples were taken within rows, between plants, to a depth of 60 cm. Each soil core was separated into three individual samples based on depth; 0-15 cm, 15-30 cm, and 30-60 cm. Once the cores were separated by depth, the three samples per plot were composited to make one sample for each depth. Samples were then dried, ground, and extracted. The residual NO₃-N and NH₄-N were determined by adding 5.00 g of soil and 50 ml of 1.0 M KCl to a plastic centrifuge tub. The samples were then agitated on an oscillating shaker for 1 hour and then spun in a centrifuge for 10-minutes at 4000 rpm. Following the spin down a 20 ml aliquot was pipetted into a scintillation vial. The vials were frozen until analyses for NO₃ and NH₄ were conducted colorimetrically on an automated ion analyzer³ which used a Cd reduction method for determining NO₃ (Bundy and Meisinger, 1994)

Cured leaf samples from four primings were composited on a weighted-mean basis by

³ QuikChem 8000, Lachat Instruments, Milwaukee, WI.
harvest from each plot for chemical analysis. Lamina was removed from the mid-vein, dried, and ground to pass through a 1-mm sieve. Total alkaloids and reducing sugars were determined using methods developed by Harvey et al. (1969). Total cured leaf yield was obtained for each plot. An Official Standard Grade was assigned to each priming, and a grade index indicating physical leaf quality, was determined for each plot (Fisher et al., 2008a). Value per hectare and value per kilogram were computed for each plot using a standard price index for each grade.

Data were subjected to ANOVA with partitioning appropriate for the factorial treatment arrangements. In the analysis, years and locations were assumed random, and nitrogen rates and sources were considered fixed. Nitrogen rates were compared using quadratic regression models using Sigma Plot⁴.

RESULTS AND DISCUSSION

Yield, Quality, and Value

In 2004, nitrogen source did not affect yield, grade index, value per hectare, value per kilogram, reducing sugars, total alkaloids, or SPAD readings (Tables 1 and 3). Therefore, all data were averaged over nitrogen source. Nitrogen rate did not affect grade index or dollars per kilogram (Tables 1 and 5). Grade index ranged from a 64 to an 80 which gave a range in dollars per kilogram from $3.48 to $3.88.

⁴ Sigma Plot. Systat Software, Inc. 1735, Technology Dr., Ste 430, San Jose, CA 95110.
There was a location by nitrogen rate interaction for yield (Table 1). There was a positive correlation between yield and nitrogen rate ($r^2 = 0.96$) in Kinston. Yield at Kinston in 2004 increased as nitrogen rate increased (Figure 1). There was also a positive correlation between yield and nitrogen rate ($r^2 = 98$) in Oxford. Yield at Oxford increased as rate increased up to 45 kg nitrogen per hectare and began to plateau (Figure 2). However, yield continued to increase as nitrogen rate increased at Kinston. Maximum yield obtained was 3362 and 3504 kg of cured leaf per hectare at the Kinston and Oxford locations, respectively.

Value per hectare increased as nitrogen rate increased (Tables 1 and 5). Value per hectare followed the same trend as yield with grade index and value per kilogram not being significant. Dollars per hectare increased as nitrogen rate increased up to 45 kg per hectare in 2004 (Table 5). Increasing nitrogen above 45 kg N/ha did not increase return per hectare. Maximum return per hectare was $12,513 at 45 kg nitrogen per hectare.

Total alkoloids and reducing sugar were effected by nitrogen rate (Tables 1 and 5). Total alkaloids increased as nitrogen rate increased up to 67 kg N/ha. Total alkaloids increased from 1.5- to 2.19-% as nitrogen rate increased from 0 to 67 kg N/ha (Table 5). Inversely, as the rate of nitrogen increased the percent total sugars decreased (Table 5). Total sugars decreased from 20.8- to 15.0-% as nitrogen rate increased from 0 to 90 kg/ha. Increasing total alkaloids and decreasing total sugars as nitrogen rate increases agrees with previous fertility research (McCants and Woltz, 1967; Elliot, 1970; Weybrew and Woltz, 1975; Link and Terrill, 1982; Weybrew et al., 1983; Court and Hendel, 1986).

SPAD readings increased as nitrogen rate increased up to 67 kg N/ha, correlating to an
increase in green leaf color with an increase in nitrogen application (Table 5). SPAD readings increased from 21.1 to 32 as nitrogen application increased from 0 to 67 kg N/ha.

In 2005 and 2006 there were no interactions or main effects of nitrogen source (Table 2 and 7). Therefore all data were pooled over nitrogen source. There were no interactions or main effects of nitrogen rate on quality index or dollars per kilogram. Quality index ranged from an 80 to 83 equating to $2.76 to $2.93 per kilogram of cured leaf.

There was a nitrogen rate main effect on SPAD readings in 2005 and 2006. SPAD Readings increased as nitrogen rate increased up to 67 kg N/ha with an increase in reading from 25.4 to 42.6 (Table 6). Increasing nitrogen rate above 67 kg/ha did not increase SPAD readings.

The relationship between yield and nitrogen rate ($r^2 = 0.99$) was positively correlated in 2005 and 2006 (Figure 3). Yield increased as nitrogen rate increased up to 67 kg N/ha. Yields plateau between 67 and 90 kg N/ha before falling with increasing rates of nitrogen. Yield was maximized at approximately 3140 kg/ha with 67 kg N/ha.

There was a nitrogen rate main effect for dollars per hectare (Table 6). Dollars per hectare followed the same trend as yield where dollars per hectare increased as nitrogen rate and yield increased. The trend between yield and dollars per hectare is due to no difference among treatments for quality index or dollars per kilogram. Treatments which received 67 kg N/ha or greater returned more dollars per hectare than did the 0 N check. Increasing the nitrogen rate above 67 kg/ha did not increase yield, therefore, dollars per hectare did not increase. Also, following the same trends as yield, 134 kg N/ha did not increase dollars per
hectare above the 0 N check.

There was a nitrogen rate main effect for total alkaloids and reducing sugars (Tables 2 and 6). Total alkaloids increased as nitrogen rate increased up to 112 kg N/ha. Total alkaloids increased from 2.01- to 3.25-percent as nitrogen rate increased from 0 to 112 kg N/ha (Table 6). Inversely, as the rate of nitrogen increased the percent reducing sugars decreased (Table 6). Reducing sugars decreased from 19.1- to 11.0-percent as nitrogen rate increased from 0 to 112 kg/ha. Increasing total alkaloids and decreasing reducing sugars as nitrogen rate increases agrees with previous fertility research (McCants and Woltz, 1967; Elliot, 1970; Weybrew and Woltz, 1975; Link and Terrill, 1982; Weybrew et al., 1983; Court and Hendel, 1986).

*Soil Nitrate and Ammonium Levels*

In 2004, soil nitrate and ammonium were not affected by nitrogen source or rate. Nitrate levels in the soil at topping ranged from 6.3 kg/ha in the upper 15-cm to 4.3 between 30 and 60-cm (Table 8). Ammonium levels ranged form 9.1 kg/ha in the upper range and decreased to 6.9 kg/ha between 30 and 60-cm. Nitrate levels in the upper 15-cm were similar at the time of final harvest. However, nitrate levels increased from an average of 5.7 kg/ha at topping to 8.8 kg/ha at final harvest (Table 9). The increase in nitrate in the 15- to 30-cm depth could be due to nitrification of ammonium. The ammonium level decreased in the upper 15-cm from an average of 9.5 kg/ha to 7.9 kg/ha between sampling at topping and final harvest (Tables 8 and 9). Therefore, the increase in nitrate nitrogen at final harvest
could have come from nitrification of the ammonium in the upper soil depth followed by some amount of leaching to the mid-sampling depth.

Since there were no significant interactions for soil data in 2005 or 2006, data were averaged over years and locations (Table 10). Nitrogen rate did effect soil nitrate and total nitrogen for both sampling times. Soil samples from treatments receiving 134 kg N/ha averaged 30.1 kg and 20.8 kg nitrate nitrogen per hectare in the upper 60-cm of soil at topping and following the final harvest respectively. Nitrate levels with the 134 kg/ha treatment were nearly double the nitrate levels of all other treatments sampled. High levels of nitrate observed with this treatment resulted in higher nitrate and ammonium levels in the soil compared with other treatments. Higher levels of residual nitrogen observed in the soil at the end of the growing season show that this treatment exceeded the nitrogen requirements for the crop. Thus, excess nitrogen is left in the soil, which has the potential to leach and contaminate water supplies.

Soil ammonium was higher for the upper 15-cm of soil at the time of topping (Table 11). Treatments receiving UAN had higher rates of ammonium than did treatments receiving calcium nitrate. Ammonium levels in the soil were 7.2- and 4.2-kg N/ha for UAN and calcium nitrate treated plots respectively. No differences were observed for nitrate levels in the upper 60-cm. The amount of nitrate in the soil ranged from 3.4- to 5.3-kg N/ha.

Soil nitrate levels, at final harvest, increased as the rate of nitrate application increased for each treatment (Table 12). Treatments receiving calcium nitrate had higher levels of nitrate in the 0-15 and 15-30 cm sampling depths. Nitrate levels in soil samples for the 0-15 cm
depth and the 15-30 cm depth was 8.2- and 6.5-kg/ha, respectively, for the treatments receiving calcium nitrate. Treatments receiving UAN had nitrate levels of 4.6- and 2.7-kg/ha at the corresponding soil depths. There were no differences between sources for levels of ammonium in the soil at final harvest. Levels of ammonium varied in the soil profile tested from 2.4- to 4.9-kg/ha.

**SUMMARY**

No differences were observed for nitrogen source on yield, quality index, dollars per hectare, total alkaloids, and total reducing sugars. Results are consistent with those previously reported by: Tisdale (1952); Shaw (1963); and Maw et al. (1995) where no differences were observed among sources of nitrogen that contained at least 50% nitrate nitrogen and those that did not. Results do not agree with research reported by McCants and Woltz (1967), who reported reduced yields and quality with nitrogen sources used for tobacco production that did not contain at least 50% of the N from a source of nitrate nitrogen. However, McCants and Woltz do say that in field situations nitrification can occur at a high enough rate that yield and quality would not be affected by a higher percent of ammonium, which is the type of environment that growers produce flue-cured tobacco in. Environment that is highly conducive to nitrification taking place, highly aerated, low to moderate soil moisture, soil pH of 5.7 to 6.0 which all are very good for soil microbial population. Therefore 30% UAN is a good source of nitrogen to use in flue-cured tobacco production.
There was a nitrogen rate effect on yield, dollars per hectare, total alkaloids, and total reducing sugars. Yield and dollars per hectare increased as nitrogen rate increased up to 45- or 67-kg N/ha at Oxford and Kinston, respectively. Quality index and dollars per kilogram were not affected by rate of nitrogen. Therefore, the increase in dollars per hectare followed the same trend as yield. Total alkaloids increased as nitrogen rate increased up to 112 kg/ha. Inversely, total reducing sugars decreased as nitrogen rate increased.

Soil nitrate levels increased at the highest level of nitrogen tested at both topping and final harvest sampling times. Nitrate levels at the two intervals for treatments receiving 134 kg N/ha were almost double that of any other treatment tested.

Ammonium levels were higher, at topping, in the 0- to 15-cm soil samples treated with UAN than treatments receiving other sources. Rates of ammonium were 7.2- and 4.2-kg/ha for UAN and calcium nitrate, respectively. At final harvest, soil nitrate levels were higher, in the upper two soil testing levels, for treatments receiving calcium nitrate. Treatments receiving calcium nitrate had 8.2- and 6.5-kg nitrate per hectare compared to 4.6- and 2.7-kg nitrate per hectare with UAN.

This information shows that any of the sources tested could be used to supply nitrogen to flue-cured tobacco. Historically, growers have used complete fertilizers blended for use in flue-cured production. These blends contained high rates of phosphorous which applied to the crop each time tobacco was grown has lead to an increase in soil phosphorous levels (data not shown). Therefore, many fields currently used to produce flue-cured tobacco do not need additional phosphorous. This study shows that on similar soils growers can apply
a potassium source at a rate that will give them 134 kg/ha of potassium and then use a source such as 30% liquid UAN for the total nitrogen source and produce the same yield and quality as they would with their traditional fertility programs. This fact is very important today as fertilizer prices have increased dramatically over the past year. By using a potassium source and a separate nitrogen source a grower can fertilize his crop for $458 per hectare compared to $1099 per hectare with traditional complete fertilizer programs. Therefore, saving the grower approximately 58 percent.


Table 1. Analysis of variance - leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars 2004 (p-value).

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<th>Source</th>
<th>Leaf Color (df)</th>
<th>Leaf Color (p-value)</th>
<th>Grade Yield (df)</th>
<th>Grade Yield (p-value)</th>
<th>Grade Index (df)</th>
<th>Grade Index (p-value)</th>
<th>Value (df)</th>
<th>Value (p-value)</th>
<th>Price (df)</th>
<th>Price (p-value)</th>
<th>Total Alkaloids (df)</th>
<th>Total Alkaloids (p-value)</th>
<th>Total Reducing Sugars (df)</th>
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<tbody>
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\( ^a \) Grade Index, Based on U.S. Government grades; 1-100, with 100 being the best.

\( ^b \) Value, Gross return per hectare (yield x price).

\( ^c \) Price, Dollars per kilogram of tobacco.
Table 2. Analysis of variance - leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars 2005 and 2006 (p-value).

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</tr>
<tr>
<td>Rep(exp)</td>
<td>12</td>
<td>0.0001</td>
<td>0.6404</td>
</tr>
<tr>
<td>Source</td>
<td>1</td>
<td>0.2977</td>
<td>0.1468</td>
</tr>
<tr>
<td>Rate</td>
<td>4</td>
<td>0.0001</td>
<td>0.1198</td>
</tr>
<tr>
<td>Source y*Rate</td>
<td>4</td>
<td>0.5702</td>
<td>0.3029</td>
</tr>
<tr>
<td>Exp*Source</td>
<td>3</td>
<td>0.2334</td>
<td>0.8410</td>
</tr>
<tr>
<td>Exp*Rate</td>
<td>12</td>
<td>0.0974</td>
<td>0.3019</td>
</tr>
<tr>
<td>Exp<em>Source</em>Rate</td>
<td>12</td>
<td>0.6657</td>
<td>0.6899</td>
</tr>
</tbody>
</table>

*a Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

*b Value, Gross return per hectare (yield x price).

*c Price, Dollars per kilogram of tobacco.
Table 3. Effect of nitrogen source averaged over locations and rates in 2004 on leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars.\(^a\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Leaf Color</th>
<th>Leaf Yield (kg/ha)</th>
<th>Grade Index (1-100)</th>
<th>Value ($/ha)</th>
<th>Price ($/kg)</th>
<th>Alkaloids (%)</th>
<th>Reducing Sugars (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea Amm. Nitrate</td>
<td>28.3 a</td>
<td>3288 a</td>
<td>73 a</td>
<td>12,170 a</td>
<td>3.70 a</td>
<td>1.91 a</td>
<td>19.0 a</td>
</tr>
<tr>
<td>Amm. Nitrate</td>
<td>28.8 a</td>
<td>3244 a</td>
<td>73 a</td>
<td>12,046 a</td>
<td>3.70 a</td>
<td>2.02 a</td>
<td>18.3 a</td>
</tr>
<tr>
<td>Calcium Nitrate</td>
<td>28.2 a</td>
<td>3329 a</td>
<td>73 a</td>
<td>12,474 a</td>
<td>3.75 a</td>
<td>2.05 a</td>
<td>17.8 a</td>
</tr>
<tr>
<td>LSD</td>
<td>2.09</td>
<td>232</td>
<td>8.8</td>
<td>1,060</td>
<td>0.15</td>
<td>0.44</td>
<td>2.3</td>
</tr>
<tr>
<td>p-value</td>
<td>0.5288</td>
<td>0.4434</td>
<td>0.9798</td>
<td>0.3852</td>
<td>0.5927</td>
<td>0.4755</td>
<td>0.2873</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at \(P = 0.05\).

\(^b\) Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

\(^c\) Value, Gross return per hectare (yield x price).

\(^d\) Price, Dollars per kilogram of tobacco.
Table 4. Effect of nitrogen rate averaged over locations and sources in 2004 on leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Rate</th>
<th>Leaf Color</th>
<th>Grade Index \textsuperscript{b}</th>
<th>Value \textsuperscript{c}</th>
<th>Price \textsuperscript{d}</th>
<th>Total Alkaloids</th>
<th>Total Reducing Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg/ha</td>
<td>1-100</td>
<td>$/ha</td>
<td>$/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21.1 d</td>
<td>64 a</td>
<td>10,085 c</td>
<td>3.48 a</td>
<td>1.50 d</td>
<td>20.8 a</td>
</tr>
<tr>
<td>22</td>
<td>24.3 cd</td>
<td>69 a</td>
<td>11,325 bc</td>
<td>3.64 a</td>
<td>1.69 cd</td>
<td>20.1 b</td>
</tr>
<tr>
<td>45</td>
<td>29.0 bc</td>
<td>74 a</td>
<td>12,513 ab</td>
<td>3.75 a</td>
<td>2.07 bc</td>
<td>18.5 c</td>
</tr>
<tr>
<td>67</td>
<td>32.0 ab</td>
<td>78 a</td>
<td>13,323 ab</td>
<td>3.81 a</td>
<td>2.19 ab</td>
<td>17.4 d</td>
</tr>
<tr>
<td>90</td>
<td>35.8 a</td>
<td>80 a</td>
<td>13,881 a</td>
<td>3.88 a</td>
<td>2.51 a</td>
<td>15.0 e</td>
</tr>
<tr>
<td>LSD</td>
<td>6.7</td>
<td>17</td>
<td>2,038</td>
<td>0.51</td>
<td>0.3817</td>
<td>0.246</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0175</td>
<td>0.2437</td>
<td>0.0296</td>
<td>0.3829</td>
<td>0.0089</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\textsuperscript{b} Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

\textsuperscript{c} Value, Gross return per hectare (yield x price).

\textsuperscript{d} Price, Dollars per kilogram of tobacco.
Table 5. Effect of nitrogen rate averaged over locations and sources in 2005 and 2006 on leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars.

<table>
<thead>
<tr>
<th>Rate kg/ha</th>
<th>Leaf Color</th>
<th>Grade Index b</th>
<th>Value c $/ha</th>
<th>Price d $/kg</th>
<th>Total Alkaloids %</th>
<th>Reducing Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25.4 c</td>
<td>81 a</td>
<td>7148 b</td>
<td>2.78 a</td>
<td>2.01 f</td>
<td>19.1 a</td>
</tr>
<tr>
<td>22</td>
<td>30.8 bc</td>
<td>80 a</td>
<td>7687 ab</td>
<td>2.76 a</td>
<td>2.23 e</td>
<td>17.5 ab</td>
</tr>
<tr>
<td>45</td>
<td>35.6 b</td>
<td>82 a</td>
<td>8460 ab</td>
<td>2.84 a</td>
<td>2.49 d</td>
<td>16.5 bc</td>
</tr>
<tr>
<td>67</td>
<td>42.6 a</td>
<td>83 a</td>
<td>9038 a</td>
<td>2.89 a</td>
<td>2.74 c</td>
<td>15.2 c</td>
</tr>
<tr>
<td>90</td>
<td>46.2 a</td>
<td>83 a</td>
<td>9169 a</td>
<td>2.93 a</td>
<td>3.04 b</td>
<td>13.1 d</td>
</tr>
<tr>
<td>112</td>
<td>47.7 a</td>
<td>82 a</td>
<td>8899 a</td>
<td>2.89 a</td>
<td>3.25 b</td>
<td>11.0 d</td>
</tr>
<tr>
<td>134</td>
<td>48.9 a</td>
<td>81 a</td>
<td>8482 ab</td>
<td>2.87 a</td>
<td>3.36 a</td>
<td>9.8 e</td>
</tr>
<tr>
<td>LSD</td>
<td>6.76</td>
<td>4.5</td>
<td>1655</td>
<td>0.2615</td>
<td>0.192</td>
<td>1.64</td>
</tr>
</tbody>
</table>

a Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

b Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

c Value, Gross return per hectare (yield x price).
Table 6. Effect of nitrogen source averaged over locations and rates in 2005 and 2006 on leaf color, yield, grade index, value, price, total alkaloids, and reducing sugars.\(^a\)

<table>
<thead>
<tr>
<th>Source</th>
<th>Grade</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Index</td>
<td>Value</td>
</tr>
<tr>
<td>Urea Amm. Nitrate</td>
<td>82 a</td>
<td>8321 a</td>
</tr>
<tr>
<td>Amm. Nitrate</td>
<td>81 a</td>
<td>8326 a</td>
</tr>
<tr>
<td>Calcium Nitrate</td>
<td>81 a</td>
<td>8586 a</td>
</tr>
<tr>
<td>LSD</td>
<td>1.6</td>
<td>246</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at \(P = 0.05\).

\(^b\) Grade Index. Based on U.S. Government grades; 1-100, with 100 being the best.

\(^c\) Value, Gross return per hectare (yield x price).

\(^d\) Price, Dollars per kilogram of tobacco.
Table 7. Effect of nitrogen source on soil nitrate and ammonium levels at topping averaged over locations and nitrogen rates in 2004.a

<table>
<thead>
<tr>
<th>Source</th>
<th>NO₃</th>
<th></th>
<th>NH₄</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-15cm</td>
<td>15-30 cm</td>
<td>30-60 cm</td>
<td>0-15cm</td>
</tr>
<tr>
<td>Calcium Nitrate</td>
<td>6.3 a</td>
<td>5.6 a</td>
<td>4.3 a</td>
<td>9.1 a</td>
</tr>
<tr>
<td>Urea Amm.</td>
<td>6.0 a</td>
<td>5.3 a</td>
<td>4.3 a</td>
<td>9.9 a</td>
</tr>
</tbody>
</table>

Nitrate

| LSD      | 1.1 | 1.1 | 0.9 | 0.9 | 0.9 | 0.7 |

| p-value  | 0.9231 | 0.9141 | 0.9849 | 0.1038 | 0.7299 | 0.9582 |

a Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

b Abbreviation: kg/ha = kilograms per hectare.
Table 8. Effect of nitrogen source on soil nitrate and ammonium levels at final harvest averaged over locations and nitrogen rates in 2004.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Source</th>
<th>NO\textsubscript{3}</th>
<th></th>
<th>NH\textsubscript{4}</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-15cm</td>
<td>15-30 cm</td>
<td>30-60 cm</td>
<td>0-15cm</td>
</tr>
<tr>
<td>Calcium Nitrate</td>
<td>6.2 a</td>
<td>7.7 a</td>
<td>3.4 a</td>
<td>7.9 a</td>
</tr>
<tr>
<td>Urea Amm. Nitrate</td>
<td>6.4 a</td>
<td>8.0 a</td>
<td>3.8 a</td>
<td>7.9 a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.9</td>
<td>1.1</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.5117</td>
<td>0.4782</td>
<td>0.5513</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\textsuperscript{b} Abbreviation: kg/ha = kilograms per hectare.
Table 9. Effect of nitrogen rate on soil nitrate and ammonium levels averaged over locations and nitrogen sources in 2005 and 2006.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg/ha</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>45</td>
</tr>
<tr>
<td>67</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>134</td>
</tr>
<tr>
<td>LSD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Topping</th>
<th>Final Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO\textsubscript{3}</td>
<td>NH\textsubscript{4}</td>
</tr>
<tr>
<td>kg/ha</td>
<td></td>
<td>kg/ha \textsuperscript{b}</td>
</tr>
<tr>
<td>0</td>
<td>15.9 b</td>
<td>14.1 a</td>
</tr>
<tr>
<td>45</td>
<td>12.1 b</td>
<td>11.3 a</td>
</tr>
<tr>
<td>67</td>
<td>14.0 b</td>
<td>12.2 a</td>
</tr>
<tr>
<td>90</td>
<td>16.9 b</td>
<td>13.3 a</td>
</tr>
<tr>
<td>134</td>
<td>30.1 a</td>
<td>13.4 a</td>
</tr>
<tr>
<td>LSD</td>
<td>9.1</td>
<td>3.9</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0012</td>
<td>0.3214</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\textsuperscript{b} Abbreviation: kg/ha = kilograms per hectare.
Table 10. Effect of nitrogen source on soil nitrate and ammonium levels at topping averaged over locations and nitrogen rates in 2005 and 2006.\(^a\)

<table>
<thead>
<tr>
<th>Source</th>
<th>NO(_3) 0-15 cm</th>
<th>NO(_3) 15-30 cm</th>
<th>NO(_3) 30-60 cm</th>
<th>NH(_4) 0-15 cm</th>
<th>NH(_4) 15-30 cm</th>
<th>NH(_4) 30-60 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Nitrate</td>
<td>4.8 a</td>
<td>4.8 a</td>
<td>3.7 a</td>
<td>4.2 b</td>
<td>4.7 a</td>
<td>4.3 a</td>
</tr>
<tr>
<td>Urea Amm. Nitrate</td>
<td>5.3 a</td>
<td>4.5 a</td>
<td>3.4 a</td>
<td>7.2 a</td>
<td>2.3 a</td>
<td>3.6 a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.9</td>
<td>0.8</td>
<td>0.9</td>
<td>3.0</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>p-value</td>
<td>0.3519</td>
<td>0.5481</td>
<td>0.4473</td>
<td>0.0001</td>
<td>0.0721</td>
<td>0.2184</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\(^b\) Abbreviation: kg/ha = kilograms per hectare.
**Table 11.** Effect of nitrogen source on soil nitrate and ammonium levels at final harvest averaged over locations and nitrogen rates in 2005 and 2006.\(^a\)

<table>
<thead>
<tr>
<th>Source</th>
<th>NO(_3) 0-15 cm</th>
<th>NO(_3) 15-30 cm</th>
<th>NO(_3) 30-60 cm</th>
<th>NH(_4) 0-15 cm</th>
<th>NH(_4) 15-30 cm</th>
<th>NH(_4) 30-60 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Nitrate</td>
<td>8.2 a</td>
<td>6.5 a</td>
<td>3.4 a</td>
<td>4.4 a</td>
<td>4.9 a</td>
<td>2.6 a</td>
</tr>
<tr>
<td>Urea Amm. Nitrate</td>
<td>4.6 b</td>
<td>2.7 b</td>
<td>2.5 a</td>
<td>4.0 a</td>
<td>3.7 a</td>
<td>2.4 a</td>
</tr>
<tr>
<td>LSD</td>
<td>1.1</td>
<td>1.5</td>
<td>1.0</td>
<td>1.1</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0013</td>
<td>0.0001</td>
<td>0.1164</td>
<td>0.7462</td>
<td>0.2344</td>
<td>0.6471</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same letter within a column are not different according to Fisher’s Protected LSD test at P = 0.05.

\(^b\) Abbreviation: kg/ha = kilograms per hectare.
Figure 1. Effect of nitrogen rate on tobacco yield at Kinston, 2004

\[ y = 2801.5 + 8.53691x - 0.0152185^2 \]

\[ (r^2 = 0.96, P = 0.038) \]
Figure 2. Effect of nitrogen rate on tobacco cured leaf yield at Oxford, 2004

\[ y = 2985.85 + 15.2629x - 0.0720054x^2 \]

\[ (r^2 = 0.98, \; P = 0.0177) \]
Figure 3. Effect of tobacco cured leaf yield at Kinston and Oxford, 2005-2006