Studies of Low-Nicotine Flue-Cured Tobacco Production

Caleb Daniel Hinkle

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T. David Reed
William H. Frame
Wade E. Thomason

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ABSTRACT

Flue-cured tobacco (*Nicotiana tabacum*) has been managed to optimize the yield of high-quality cured leaf while maintaining nicotine levels within a relatively narrow range based on the Regional Minimum Standards Program. Among the 3,000 plus alkaloids found in tobacco, nicotine accounts for greater than 90% of the total alkaloids produced in commercial tobacco varieties. Precious research has demonstrated an association with nicotine levels and cured leaf yield and quality. On March 16, 2018, the Food and Drug Administration issued a notice for proposed rulemaking to limit nicotine in combustible cigarettes to 0.3-0.5 mg nicotine per gram of tobacco (an approx. 98% reduction from current levels). Studies on achieving decreased levels of nicotine in flue-cured tobacco have been conducted since the mid-1900's and some success has been found through breeding and genetics. The FDA proposal suggested changes to standard agronomic production practices as a means of achieving their proposed nicotine levels. The primary objectives of this work are: (1) evaluate the impact of standard agronomic production practices on nicotine levels and the resulting yield and cured leaf quality and (2) quantify differences in nitrogen-use efficiency between conventional and low-nicotine flue-cured tobacco varieties. Results showed that the changes to production practices did not sufficiently lower nicotine levels to the range proposed by the FDA. The only publicly available low-nicotine variety, LA FC53, did not achieve the targeted nicotine level and the yield and cured leaf quality was severely reduced. The study of nitrogen-use efficiency quantified differences between conventional and low-nicotine flue-cured tobacco varieties. Proprietary, low-nicotine varieties developed from K 326 tended to have lower nicotine levels than LA FC53 with improved yield and cured leaf quality. Both yield and quality are associated with nitrogen-use efficiency and the nitrogen-use efficiency of the new low-nicotine lines was comparable to K 326 and better than the previously developed, low-nicotine standard variety. No evaluation of smoking characteristics of the cured tobacco from these studies was conducted and would be necessary for commercial utilization of low-nicotine flue-cured tobacco.

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General Audience Abstract

Flue-cured tobacco (*Nicotiana tabacum*) has been managed to optimize the yield of high-quality cured leaf while maintaining nicotine levels within a relatively narrow range based on the Regional Minimum Standards Program. Among the 3,000 plus alkaloids found in tobacco, nicotine accounts for greater than 90% of the total alkaloids produced in commercial tobacco varieties. Precious research has demonstrated an association with nicotine levels and cured leaf yield and quality. On March 16, 2018, the Food and Drug Administration issued a notice for proposed rulemaking to limit nicotine in combustible cigarettes to 0.3-0.5 mg nicotine per gram of tobacco (an approx. 98% reduction from current levels). Studies on achieving decreased levels of nicotine in flue-cured tobacco have been conducted since the mid-1900's and some success has been found through breeding and genetics. The FDA proposal suggested changes to standard agronomic production practices as a means of achieving their proposed nicotine levels. The primary objectives of this work are: (1) evaluate the impact of standard agronomic production practices on nicotine levels and the resulting yield and cured leaf quality and (2) quantify differences in nitrogen-use efficiency between conventional and low-nicotine flue-cured tobacco varieties. Results showed that the changes to production practices did not sufficiently lower nicotine levels to the range proposed by the FDA. The only publicly available low-nicotine variety, LA FC53, did not achieve the targeted nicotine level and the yield and cured leaf quality was severely reduced. The study of nitrogen-use efficiency quantified differences between conventional and low-nicotine flue-cured tobacco varieties. Proprietary, low-nicotine varieties developed from K 326 tended to have lower nicotine levels than LA FC53 with improved yield and cured leaf quality. Both yield and quality are associated with nitrogen-use efficiency and the nitrogen-use efficiency of the new low-nicotine lines was comparable to K 326 and better than the previously developed, low-nicotine standard variety. No evaluation of smoking characteristics of the cured tobacco from these studies was conducted and would be necessary for commercial utilization of low-nicotine flue-cured tobacco.

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Chapter I

Introduction

Flue-cured tobacco is an important cash crop in the piedmont and coastal plain region of the southeast United States with the bulk of production in North Carolina. Among all tobacco types produced in the United States in 2020, flue-cured accounted for roughly 65% of the tobacco produced nationwide (USDA, 2021). The lowest acreage of flue-cured tobacco ever produced in Virginia was in 2020. Flue-cured tobacco has historically been the most valuable field crop grown in the commonwealth but production has declined in recent years.

Alkaloids are nitrogen-containing organic bases found in over 4,000 plant species and nicotine (Figure 1.1) is one of the most well-known among the total 3,000 plus alkaloids (Kurek, 2019). These compounds have a wide array of physiological effects in humans and biological effects in plants. Nicotine functions as a defense mechanism against pests in tobacco (Tso, 1990; Preisser *et al.*, 2007). Among the total alkaloid level in tobacco, nicotine accounts for greater than 90% and the manipulation of nicotine levels can cause other alkaloid levels to rise and fall (Tso, 1990; Benowitz *et al.*, 2009; Dewey & Xie, 2013). The lowering of nicotine levels in tobacco has been a research topic since the 1960’s (Wayne, 2012). Numerous techniques such as breeding, genetic modification, and chemical extraction have been experimented to reduce nicotine levels in tobacco both in-situ and post-harvest (FDA, 2018).

Tobacco has been managed for many years to increase yield and quality while maintaining nicotine levels within a relatively narrow range for commercial use, based on the Regional Minimum Standards Program (Bowman, 1996). Nicotine levels have been shown to be directly related to the grade, yield, total nitrogen, and chlorophyll levels in flue-cured tobacco varieties (Chaplin & Weeks, 1976). Low-nicotine flue-cured tobacco is characterized by both lower yield and cured leaf quality (Chaplin & Burk, 1984). Standard cultural practices such as fertilization, topping, plant spacing, and sucker control have been established over time to maximize tobacco production. On March 16, 2018, the Food and Drug Administration issued a new proposed nicotine level for cigarette products to be between 0.3-0.5 mg nicotine per gram of tobacco as compared to the current range of 14-27 mg nicotine per gram of tobacco (FDA, 2018). Lowering nicotine levels in tobacco products has been an interest since the mid-1900s and some success has been achieved through breeding and genetics (Dewey & Xie, 2013). The issues with altering agronomic practices to produce reduced nicotine levels can greatly affect many factors of production (Murillo, 2018). Numerous experiments have been conducted, resulting in no agronomic plan that can consistently produce nicotine levels to support the range desired by the FDA while maintaining yield and quality.

Changes in fertilization rates, topping schedules, topping heights, sucker controls, irrigation rates, plant spacing (plant population), and harvest techniques are being tested to potentially develop new agronomic production plans that maintain the desired quality and yield of flue-cured tobacco while complying to the potential nicotine reduction levels (Henry *et al.*, 2019). Studies have also been conducted to observe these effects on low nicotine varieties and how they compare to conventional lines (Chaplin & Burk, 1977). LAFC 53 is the only publicly available low-nicotine variety and has been the main constituent used in the testing of these effects. As well as LAFC 53, other low-nicotine varieties have yet to efficiently achieve the proposed standards from the FDA (Lewis *et al.*, 2020). Studies with increased plant populations have reduced nicotine levels by one-third (Collins & Hawks, 1983); however, this level does not meet the desired FDA range of approximately 98% reduction (Murillo, 2018). In addition to population evaluations, topping studies were evaluated to determine the effectiveness of not topping plants at all. Topping is a standard commercial production practice to increase yield, reduce insect damage, stabilize plants, and stimulate root development to increase the efficiency of fertilizer uptake, drought tolerance, and total alkaloid production (Fisher *et al.*, 2016). Even though topping has shown to increase nicotine in tobacco, removal of this step reduced levels by only 37% (Collins & Hawks, 1983). Studies have also shown adverse effects on the final harvest with reductions in yield, quality, and income for non-topped tobacco (Murillo, 2018). Another area of interest to reduce nicotine concerns the tobacco curing process. Changing the curing process to potentially lower nicotine levels has revealed only slight and insignificant decreases in nicotine that are still relatively similar to levels found in field-grown tobacco (Gravely *et al.*, 1973; Murillo, 2018). With the inability to effectively reduce nicotine, altering the curing process also drastically effects the product quality related to flavor and other attributes that customers desire. This combination of negative effects on flue-cured tobacco can cause a spiraling effect of larger economic issues.

Production practices have shown repeatedly to have an effect on nicotine levels in tobacco and under certain manipulations, these levels can be either reduced or increased. More focused research should be done to specialize production practices for the reduction of nicotine levels and the maintenance of yield and quality. The relationship between these factors has proven to be very problematic to obtain. With some decrease in nicotine levels in almost every step of production that was altered, results could suggest that with specific trials, an almost adequate reduced level of nicotine could be reached. Reaching this point of agronomic production will require extensive research to develop the most optimal fertilizer rates, topping schedule/height, sucker control, irrigation rate, population, and harvest technique. In response to the desired research, a two-year study was conducted at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center outside of Blackstone, VA. The objectives of this research were to:

1) Evaluate the impact of standard agronomic practices on nicotine levels and the resulting yield and cured leaf quality.
2) Quantify differences in nitrogen-use efficiency between conventional and low-nicotine flue-cured tobacco varieties.

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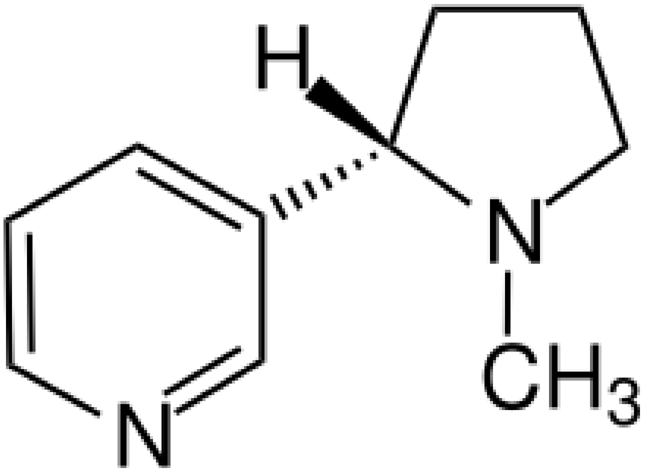

Figure 1.1. Structure of nicotine (Jacob et al., 2002).

Chapter II

Literature Review

Alkaloids in Tobacco

Alkaloids are nitrogen-containing, organic bases found in over 4,000 plant species and nicotine (Figure 2.1b) is one of the most well known among the total 3,000 plus alkaloids (Kurek, 2019). These compounds have a wide array of physiological effects in humans and biological effects in plants. Nicotine is an addictive compound and has been found comparable to hard drugs and alcohol (Stolerman and Jarvis, 1995). Tobacco products are primarily used for the desired stimulating effects they have on the user but have also been show to enhance cognitive performance in both smoking and non-smoking populations (Kumari *et al.*, 2003). As for the function of nicotine formation in tobacco, it has been suggested that its purpose is to protect the plant against insect pests and herbivores (Tso, 1990; Preisser *et al.*, 2007).

Of the genus *Nicotiana*, nicotine is the principal alkaloid in 50 to 60% of all species (Bush, 1999). There are many other alkaloids found in tobacco, but only three that are considered to be significant other than nicotine. These are described as secondary alkaloids and consist of nornicotine (Figure 2.1d), anabasine (Figure 2.1a), and anatabine (Figure 21c) (Dewey & Xie, 2013). Nornicotine is the principal alkaloid in 30 to 40% of all species while anabasine is the principal alkaloid in only four and anatabine in just three (Bush, 1999). Among the naturally occurring alkaloids in *Nicotiana tabacum*, nicotine accounts for greater than 90% and is the source for the commercial value of the crop (Tso, 1990; Benowitz *et al.*, 2009; Dewey & Xie, 2013).

Impact of Agronomic Production Practices on the Nicotine Content of Flue-Cured Tobacco

Production practices, weather, and soil fertility (most influentially nitrogen) have been shown to directly impact nicotine levels in cured tobacco (Collins & Hawks, 1983). Tobacco has been managed for many years to increase yield and quality while maintaining nicotine levels within a relatively narrow range for commercial use, based on the Regional Minimum Standards Program (Bowman, 1996). Nicotine levels have been shown to have a direct relationship to the grade, yield, total nitrogen, and chlorophyll levels in flue-cured tobacco varieties (Chaplin & Weeks, 1976). Under lower levels of nicotine, and total alkaloids, flue-cured tobacco has a much lower grade and producibility (Chaplin & Burk, 1984). Standard cultural practices such as fertilization, topping, plant spacing, and sucker control have been adjusted over time to maximize tobacco production.

The U.S. Food and Drug Administration, on March 16, 2018, issued a preproposal for new nicotine levels in cigarette products to be between 0.3-0.5 mg nicotine per gram of tobacco as compared to the current range of 14-27 mg nicotine per g (FDA, 2018). Changing the level of nicotine can cause other alkaloid levels to rise or fall, and the lowering of nicotine levels in tobacco has been a research topic since the 1960's (Wayne, 2012). The FDA cited numerous techniques such as breeding, genetic modification, and chemical extraction that have been experimented to reduce nicotine levels in tobacco both in-situ and post-harvest (FDA, 2018). One of the greatest interests in methods of nicotine reduction is the alteration of production practices (FDA, 2018). Such practices include plant spacing, row spacing, topping, nitrogen applications, irrigation applications, etc. Genetics and breeding have reduced nicotine levels in tobacco and developed new varieties for research purposes (Dewey & Xie, 2013). Countless trials have shown the weak agronomic productivity of these low-nicotine varieties at a commercial scale (Chaplin & Burk, 1984). With the difficulty to produce quality tobacco for lower levels of nicotine, these varieties can have a large, negative economic impact on farming operations (Ramsey, 2019). Altering agronomic practices to produce reduced nicotine levels in even conventional varieties can greatly affect many factors of production (Murillo, 2018).

Altered fertilizer rates, topping schedules, topping heights, sucker controls, plant/row spacing (population), and harvest techniques have been studied to maintain the desired quality and yield of flue-cured tobacco while complying to the potential nicotine reduction levels (Henry *et al.*, 2019). Studies have also evaluated these effects on low nicotine varieties and how they compare to conventional lines (Chaplin & Burk, 1977). LAFC 53 is the only publicly available low-nicotine variety and has been the main constituent used in the testing of these effects. In addition to LAFC 53, other low-nicotine breeding lines have yet to efficiently achieve the proposed standards from the FDA (Lewis *et al.*, 2020). Studies with increased plant populations have reduced nicotine levels by one-third (Collins & Hawks, 1983); however, this level does not meet the desired FDA range of approximately 98% reduction (Murillo, 2018). Along with population, topping studies have been done to evaluate the effectiveness of removing this practice from production. Topping is a standard commercial production practice to increase yield, reduce insect damage, stabilize plants, and stimulate root development (this increases the efficiency of fertilizer uptake, drought tolerance, and total alkaloid production) (Fisher, 2016). Even though topping has shown to increase nicotine in tobacco, removal of this step reduced levels by only 37% (Collins & Hawks, 1983). This study also showed adverse effects on the final harvest with significant reductions in yield, quality, and income for non-topped tobacco (Murillo, 2018). Another area of interest to reduce nicotine concerns the tobacco curing process. Changing the curing process to potentially lower nicotine levels has revealed only slight and insignificant decreases in nicotine that are still relatively similar to levels found in tobacco cured by modern production standards (Murillo, 2018). With the inability to effectively reduce nicotine, altering the curing process also drastically effects the product quality related to flavor and other attributes that customers desire. This can cause a spiraling effect of larger economic issues.

N-Use Efficiency of Low-Nicotine Flue-Cured Tobacco

For over 100 years now, flue-cured tobacco yields have substantially increased due to advancements in cultivars, production practices, and technology (Sisson *et al.*, 1991). A large improvement to the commercial production of tobacco was the implementation of increased nitrogen fertilizer rates. Prior to 1940, minimal nitrogen fertilizer was applied and rates below 35 lbs/ac eventually increased to as high as 98 lbs/ac (Sisson *et al.*, 1991). Nitrogen fertilizer research has resulted in recommendation levels between 50 and 80 lbs/ac depending on soil type and topsoil depth (Reed *et al.*, 2018). The lower range of N is recommended for a more fine-textured and shallow topsoil while the upper levels of this range are recommended for a more coarse-textured and deep topsoil (Collins & Hawks, 1983). Cropping history is a key part in determining a nitrogen fertilizer program for the upcoming year. Tobacco grown behind leguminous crops or cover crops that have been plowed into the soil are expected to leave behind nitrogen that can be used by the crop (Collins & Hawks, 1983). The most important macronutrient in the production of flue-cured tobacco is nitrogen and adequate levels are required to produce high-yielding, quality leaf (Drake *et al.*, 2015). With increased rates of nitrogen fertilizers and the development of new varieties inherently came an overall improvement of nitrogen-use efficiency (Sisson *et al.*, 1991).

Fully understanding nitrogen-use efficiency (N-USE) requires understanding its constituents: nitrogen accumulation (N-ACC), nitrogen uptake (N-UPT), nitrogen utilization (N-

UTL), and total nitrogen (Total N). Many factors that apply to these components can vary the results of nitrogen-use efficiency calculations (Morris, 1993). Issues with nitrate assimilation and reduction could affect N-UPT, while absorption of nitrogen may limit N-ACC (Dhugga and Waines, 1989). Whatever causes different types of plants or species to vary in nitrogen-use efficiency is uncertain and up for much debate (Morris, 1993). Within classes of tobacco, nitrogen-use efficiency varies (Crafts-Brandner *et al.*, 1987). Burley tobacco has a much lower nitrogen utilization as compared to flue-cured tobacco, which has the ability to produce approximately twice the dry weight per unit of accumulated leaf nitrogen (Legg *et al.*, 1977; Moll *et al.*, 1982). Efficient uptake by the root system, nitrogen translocation, and metabolic utilization of nitrogen within the plant regulate nitrogen-use efficiency (Morse & Kovach, 1981). It has been shown that the physiology of the root system is not the influence of this difference but the genetic differences at two loci, and potentially the higher content of starch in flue-cured plants, that make them more efficient users of nitrogen (Crafts-Brandner *et al.*, 1987).

When nitrogen rates are increased above a certain level there is a decrease in N-USE, N-UPT, N-UTL, and even net returns (Moll *et al.*, 1982; Sisson *et al.*, 1991). Additional, not recommended nitrogen applications, can result in lower yields and poorer quality tobacco (Morris, 1993). Even cultivars with similarly high levels of N-USE efficiencies may differ in the way in which efficiency is attained (Moll *et al.*, 1982). Morris et al. (Morris, 1993) describes this using an example of two cultivars having comparable N-USE that both produce the same yield with the first cultivar taking up all applied nitrogen (having a high N-UPT) and the second taking up only a fraction of the applied nitrogen (having a low N-UPT) resulting in a high N-UTL of the second cultivar. It is suggested that when selecting more efficient cultivars, N-UPT is the most effective characteristic for improving overall N-USE (Dhugga & Waines, 1989). In a study done by Sisson et al. (Sisson *et al.*, 1991), N-UTL accounted for only 39-45% of the variance in N-USE while N-UPT accounted for 55-61%. It is possible that the traditionally low nitrogen environment in which flue-cured tobacco is grown, has given little reason for it to develop a more efficient N-UTL (Morris, 1993).

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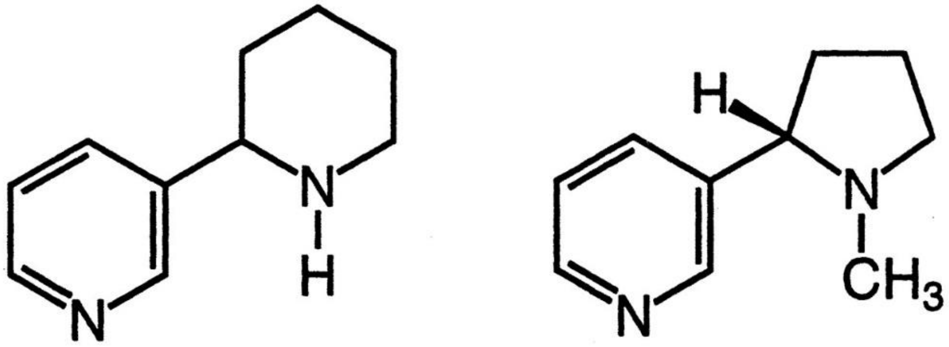
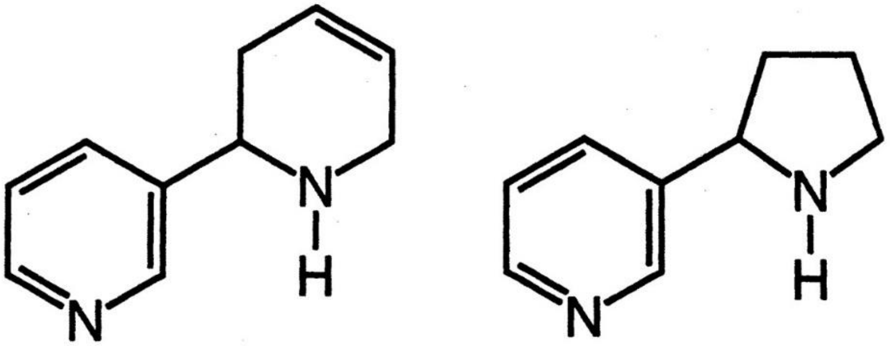


a) Anabasine b) Nicotine



c) Anatabine d) Nornicotine

Figure 2.1 a-d. Chemical structure of anabasine (a), nicotine (b), anatabine (c), and nornicotine (d) (Jacob et al., 2002).

Chapter III

Impact of Agronomic Production Practices on the Nicotine Content of Flue-Cured Tobacco

Introduction

The U.S. Food and Drug Administration (FDA) in 2018 issued a preproposal for nicotine levels in cigarette products to be between 0.3-0.5 mg nicotine per g of tobacco (FDA, 2018). The current range of nicotine in cigarette products is 14 to 27 mg nicotine per g of tobacco. This proposed change of a 98% reduction in nicotine levels could result in levels of secondary alkaloids that impact the smoking quality of the tobacco and lead to the formation of cancer causing tobacco specific nitrosamines (Wayne, 2012).

Tobacco has been managed over many years to increase yield and quality while maintaining nicotine levels within a relatively narrow range for commercial use based on the Regional Minimum Standards Program (Bowman, 1996). To obtain these industry standards, cultural practices such as fertilization, topping, plant spacing, and sucker control have been somewhat standardized over time. Nicotine levels have been shown to have a direct relationship to the grade, yield, total nitrogen, and chlorophyll in flue-cured tobacco (Chaplin & Weeks, 1976).

The FDA proposed several alternative production practices to achieve their proposed nicotine levels. The objective of the research described herein was to evaluate the impact of modifications to standard agronomic practices on the nicotine levels of a standard commercial variety and a low-nicotine variety and to quantify the impact on yield and cured leaf quality.

Materials and Methods

Three trials were conducted at the Southern Piedmont Agricultural Research and Extension Center near Blackstone, VA during the 2019 and 2020 growing seasons to evaluate production practices intended to lower nicotine levels in flue-cured tobacco. Tests included: (1) Plant Population Influence on Nicotine Production, (2) Topping Time Influence on Nicotine Production, (3) Topping Height Influence on Nicotine Production. Each of these tests were planted with two varieties: K 326 (widely grown, commercial variety) and LA FC53 (publicly available low alkaloid variety, not commercially grown). All of these tests compared the recommended nitrogen fertilization rate of 72 lbs N/ac to a reduced rate of 42 lbs N/ac. Tobacco was planted on raised beds spaced 48 in. apart and 40 ft. long. All plots were fertilized at formation of the beds prior to transplanting with 700 lbs/ac of 0-6-18 tobacco grade fertilizer. Shortly after transplanting, 12.9 gal of UAN-30 was applied for all plots to achieve a rate of 42 lbs N/ac. Immediately following this application, an additional 9.3 gal of UAN-30 was applied in specific plots to achieve the standard rate of 72 lbs N/ac. Applications of UAN-30 were made using a two-row JohnBlue squeeze pump injection rig (JohnBlue Company, Huntsville, AL). All other agronomic practices followed recommendations from the 2019 Flue-Cured Tobacco Production Guide (Reed *et al*., 2018). Pre-topping suckers were removed and plants were treated with a 4% (V/V) solution of Off-Shoot-T (a mixture of C_8, C_{10}, and C_{12} fatty alcohols) (Chemtura Corporation, Middlebury, CT). Additional applications were made immediately after topping, 5 days later, and 12 days later using a 5% solution. One week prior to harvest, a single application of Prime+ EC (flumetralin) was made at a rate of 2% (V/V) (Syngenta Crop Protection, Greensboro, NC). The plant population study evaluated the following treatments:

- 5940 plants per acre (22 in. plant spacing)
- 6534 plants per acre (20 in. plant spacing)
- 7260 plants per acre (18 in. plant spacing)
- 8168 plants per acre (16 in. plant spacing)

The topping time study involved topping or the removal of the apical bud, when plants reached a specific growth stage or crop stage. Flower development was allowed to proceed in plants for the no topping treatment, though this is not a normal practice for tobacco in the U.S. The topping time study evaluated the following treatments:

	Topping Dates	
Treatment	2019	2020
25% bloom or less	8 July	13 July
100% bloom	15 July	20 July
at first harvest	1 August	11 August
No Topping	-	-

Optimal leaf number per acre is 110,000-120,000 leaves for a plant population of 5,940 topped at 19-20 leaves high. The topping heights evaluated were 16, 19, and 22 leaves high.

Tobacco in all three tests was harvested four times as leaves ripened, beginning at the bottom of the stalk and progressing upwards. Yield is calculated as the sum of the individual harvest weights. All cured leaf was graded by a USDA AMS flue-cured tobacco grader and corresponding grade index value assigned according to Bowman et al. (1988). An overall or composite grade index value was calculated for each plot based on the proportion of the total yield represented by each harvest and their corresponding yield weights. Composite nicotine and reducing sugars were calculated in a similar fashion. Nicotine and reducing sugar levels were determined using a FOSS-5000® NIR (FOSS Analytical, Eden Prairie, MN).

Each study was analyzed as a split-split plot design with N rate as the whole plot variable, the variable of interest (population, topping time, or topping height) as the split-plot, and variety as the split-split-plot variable. Treatments were replicated four times in each field. Analysis of variance was run using SAS® version 9.4 (SAS Institute, 2019) and significant differences determined using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Plant Population Influence on Nicotine Production

Cured Leaf Chemistry

The two growing seasons were significantly different ($p < 0.0001$) in nicotine and reducing sugar levels but plant population did not have a significant effect (Table 3.1). Variety and N rate had a significant effect on the levels of nicotine and reducing sugars in both years. Nicotine was impacted significantly by an interaction of year X variety ($p < 0.0001$). An increase in plant population in 2019 had no significant effect on nicotine levels in either variety (Table 3.2). The low-nicotine LA FC53 did have a highly significant impact on nicotine at both N rates. Nicotine levels tended to decrease slightly in both varieties as the N rate was reduced below 72 lbs N/ac. Reducing sugars were not impacted by an increase of population in either variety. A decreased N rate had higher levels of reducing sugars than that of the standard and this trend was the same for both varieties. Variety LA FC53 was much lower in reducing sugars than K 326 at each treatment level. There was no significant effect on nicotine levels by an increase in population for either variety in 2020. Variety LA FC53 was much lower than K 326 in nicotine averaged across all treatments. As N rates were decreased, both varieties decreased slightly in nicotine levels. Population had no significant effect on the levels of reducing sugars in either variety. A lower N rate consistently resulted in higher levels of reducing sugars in both varieties with LA FC53 having the lowest at each treatment level.

The nicotine levels, as expected, of LA FC53 were substantially lower than the commercially grown K 326 over both years of data (Figure 3.1a). Increased plant population had no significant effect on the nicotine production of either variety. At both N rates, LA FC53 was significantly lower in nicotine than K 326. A decrease in N rate had no significant effect on the resulting nicotine levels of either variety. Reducing sugar levels averaged across all treatments were much higher in K 326 than LA FC53 (Figure 3.1b). Levels of reducing sugars in cured leaf were not impacted by the increasing population for either variety.

Cured Leaf Yield and Quality

Year was highly significant to the yield and grade index of these varieties ($p < 0.0001$) (Table 3.3). Yield and grade index were significantly impacted by variety and N rate but only yield was affected by plant population. There was a significant effect on grade index from an interaction of N rate X plant population ($p < 0.0001$). Yield of all plant populations and N rates were significantly lower with LA FC53 than K 326 in 2019 (Table 3.4). Differences were only observed in K 326 at a low N rate, with yield being significantly higher at a population of 8,168 plants/ac. Yield of all plant populations at a standard N rate of 72 lbs N/ac were substantially higher with K 326 than those found at a lowered N rate. Significant differences between plant populations were only found for LA FC53 at a standard N rate, with yield being significantly lower at the lowest population. Cured leaf quality was significantly different in both varieties at the standard N rate and was highest at an increased population. Composite grade index of LA FC53 was significantly lower at all treatment levels than K 326. An increase in population had significant effects on the yield of both varieties in 2020. Varieties LA FC53 and K 326 were both significantly lower in yield at the standard populations; however, LA FC53 was much lower than K 326. Decreasing N rates also tended to lower yield at each population for LA FC53. Yield of LA FC53 was much lower than K 326 at every treatment level. Cured leaf quality showed no significant impact by a change in population or N rate for either variety. Composite grade index of LA FC53 was significantly lower than that of K 326.

Data combined and analyzed over both the 2019 and 2020 growing seasons showed LA FC53 to have a much lower yield than K 326 (Figure 3.2a). Both varieties consistently have a higher yield at each population increase. At both N rates, LA FC53 was significantly lower than K 326. Cured leaf quality was significantly lower in LA FC53 than K 326 at every treatment level (Figure 3.2b). As N rate was increased, both varieties tended to have a lower grade index. Variety LA FC53 was significantly lower in quality than K 326 at all treatment levels.

Topping Time Influence on Nicotine Production

Cured Leaf Chemistry

Nicotine and reducing sugars were significantly impacted by year and variety (Table 3.5). Reducing sugars were also significantly affected by N rate, topping time, and an interaction of year X topping time ($p <0.0001$, $p < 0.0001$, and $p = 0.0383$, respectively). An extended topping time in 2019 had a significant effect on nicotine levels in both varieties (Table 3.6). Nicotine levels in both varieties decreased as topping was delayed or not done and were significantly lower in plants that were not topped at all. Topping time had no significant effect on reducing sugar levels of K 326. Variety LA FC3 had much higher levels of reducing sugars when the N rate was lowered. Reducing sugar levels were only significantly different when plants were topped at 1st harvest. Levels of reducing sugars were much lower in LA FC53 than K 326. Nicotine levels were significantly impacted by the topping time treatments in 2020. Variety K 326 at both N rates, was significantly lower in nicotine when plants were not topped at all as compared to the other treatment levels. At a standard N rate, LA FC53 was significantly lower in nicotine when plants were not topped at all. Averaged over all treatments, LA FC53 was significantly lower in nicotine than K 326. Cured leaf quality was significantly affected by topping time in both varieties. The highest levels of reducing sugars in both varieties were found in plants topped at 1st harvest with LA FC53 having the lowest at each treatment level. Both N rates resulted in LA FC53 having much lower reducing sugar levels than K 326. Levels of reducing sugars were consistently higher at a decreased N rate in both varieties.

Nicotine levels averaged across all treatments were significantly lower in LA FC53 than K 326 for both years (Figure 3.3a). A delay in the time of topping tended to result in a slight decrease in nicotine levels while plots not topped at all had the lowest nicotine levels among all treatments. Levels of nicotine showed no consistent change in either variety as N rate was decreased. Over both growing seasons, reducing sugar levels of LA FC53 tended to be much lower than that of K 326 (Figure 3.3b). An extended time of topping had a significant effect with levels trending upward then declining sharply when plots where not topped at all. Rate of N had a significant effect causing each variety to increase in sugar levels as the rate was lowered.

Cured Leaf Yield and Quality

Yield was significantly impacted by year, variety, and topping time ($p < 0.0001$, $p < 0.0001$, and $p = 0.0131$, respectively) (Table 3.7). Effects from year, variety, and N rate significantly impacted grade index ($p < 0.0001$, $p < 0.0001$, and $p = 0.0021$, respectively). Significant interactions of year X N rate, year X topping time, and variety X N rate were observed on grade index. Topping time treatments had a significant effect on yield for both varieties in 2019 (Table 3.8). Variety K 326, with both N rates, was significantly lower in yield when plants were not topped at all as compared to the other treatments. Similarly, the yield of LA FC53 under both N rates was significantly lower in plants that were not topped at all. An overall trend for both varieties was a decrease in yield as topping time was lengthened but significantly dropped in plants that were not topped at all. Composite grade index at each treatment level shows LA FC53 to have a significantly lower quality than K 326. Quality for both varieties was not affected much by the decreased N rate; however, both varieties tended to decrease in quality as topping time was extended. The lowest composite grade index values for both varieties were consistently found in plants that were not topped at all. Overall, both varieties tended to have the lowest quality cured leaf come from plants that were not topped at all but LA FC53 was still significantly lower than K 326 at every treatment level. Yield was significantly impacted by the topping time treatments in 2020 with an extended topping time decreasing yield in both varieties. In both varieties, an extended topping time significantly decreased yield with the lowest values found in plants not topped at all. In most treatment levels, a decreased N rate tended to lower yield in both varieties. Overall, LA FC53 was significantly lower in yield at each treatment level compared to K 326. Cured leaf quality was not impacted by topping time treatments in either variety; however, LA FC53 was significantly lower overall in composite grade index than K 326.

Yield data averaged over the two growing seasons and treatment levels shows LA FC53 to be much lower than K 326 (Figure 3.4a). Time of topping had a significant effect on yield among both years with both varieties tending to decrease under an extended topping time and the lowest being plants not topped at all. Variety LA FC53 was lower in yield at every treatment level compared to K 326. Both varieties showed a consistently higher yield at a standard N rate. Time of topping had no consistent effect on cured leaf quality in either variety (Figure 3.4b). A decrease in N rate tended to have an increase of quality in both varieties. Cured leaf quality over the two seasons was significantly lower in LA FC53 than K 326 at every treatment level.

Topping Height Influence on Nicotine Production

Cured Leaf Chemistry

Nicotine and reducing sugars were significantly impacted by year, variety, and topping height (Table 3.9). Reducing sugars were also impacted by N rate ($p = 0.0350$). Interactions of year X variety and year X topping height also had significant effects on nicotine levels. Altering topping height had no significant effect on the nicotine levels of either variety in 2019 (Table 4.10). Variety LA FC53 is much lower in nicotine at every treatment level compared to K 326. Topping height had no significant effect on reducing sugar levels in either variety but were slightly higher when the N rate was decreased. Averaged over all treatments, LA FC53 was much lower in reducing sugars than K 326. Similar to 2019, topping height had no significant effect on nicotine levels of either variety in 2020. Nicotine levels averaged across all treatments were significantly lower in LA FC53 than K 326. Reducing sugar levels were only significantly impacted by topping height in LA FC53 at a standard N rate. Plants topped at 16 leaves high were significantly lower in reducing sugars than the standard and increased topping height. Topping height had no other significant effects in either variety. Overall, LA FC53 was significantly lower in reducing sugars than K 326.

Nicotine levels averaged over all treatment levels and both seasons were significantly lower in LA FC53 than K 326 (Figure 3.5a). Both varieties slightly increased in nicotine levels as the height of topping was raised from the standard. Inversely, each variety decreased slightly in nicotine levels as topping height was lowered from the standard. Nicotine levels increased only slightly as N rate was lowered in both varieties. Reducing sugar levels combined over the two seasons were much lower in LA FC53 than K 326 (Figure 3.5b). In both varieties, reducing sugars tended to decrease slightly when topping height was lowered.

Cured Leaf Yield and Quality

Yield and grade index were significantly impacted by year, variety, topping height, and interaction of year X variety (Table 4.11). An interaction of year X topping height had a significant effect on grade index as well (p = 0.0135). The yield of both varieties was significantly impacted by topping height in 2019 (Table 3.6). Variety LA FC53 was significantly lower in yield than K 326 at each treatment level. Yield decreased with a lower topping height in both varieties but was significantly lower at a height of 16 leaves. At almost every treatment level in both varieties, yield tended to decrease when the N rate was lowered. Data averaged across all treatments show LA FC53 to have a much lower yield than K 326. Cured leaf quality was not significantly impacted by a change in topping height. Across all treatments, LA FC53 was significantly lower in composite grade index than K 326. Yield in 2020 was significantly impacted by topping height in both varieties, as seen similarly in 2019. A decreased topping height was significantly lower than the other treatment levels in both varieties with LA FC53 consistently being lower than K 326. A decreased N rate slightly lowered yields in some topping height treatments for both varieties. Averaged across all treatments, yield was much lower in LA FC53 than K 326. Again, cured leaf quality was not significantly affected by an altered topping height in either variety. Variety LA FC53 was significantly lower in composite grade index than K 326 at every treatment level.

Data combined over both growing seasons showed LA FC53 to have a much lower yield than K 326 at every treatment level (Figure 3.6a). Both varieties under each N rate decreased in yield as the topping height was lowered from the standard. An increase in topping height had no major impact on the yield of either variety. Quality was significantly lower in LA FC53 than K 326 at every treatment level (Figure 3.6b). Topping height as well as a decrease in N rate had no major effect in cured leaf quality for either variety.

Conclusions

The use of LA FC53 was the single most important factor in reducing nicotine levels in tobacco grown under a range of production practices. Of the 22 total treatment combinations for LA FC53 over the three tests reported herein: two treatments (no topping at 42 and 72lbs N/ac) in 2019 and all 22 treatments in 2020 resulted in nicotine levels to be below the proposed limit of 0.5 mg of nicotine per g of tobacco. These results highlight the impact of growing season on nicotine levels in the cured tobacco. Nicotine in the commercially grown K 326 was reduced by a lowered N fertilization and other production practices but the resulting nicotine levels averaged seven times the proposed FDA standard.

Lower cured leaf nicotine levels achieved with the use of LA FC53 came at the great expense of yield and quality of the cured tobacco. The yield of LA FC53 averaged 23% less than K 326 with standard production practices and was reduced further by reducing N fertilization and not topping plants to further reduce nicotine production. No cured tobacco from any treatment with LA FC53 received a marketable grade.

The proposed FDA standard of 0.3-0.5 mg nicotine per g of tobacco would be difficult to consistently achieve with the currently available low-nicotine flue-cured tobacco variety (LA FC53). Changing our standard production practices to either reduce nicotine synthesis (increased plant population) or dilute nicotine translocation to the harvested leaves (topping height and topping time) were shown to have a negative impact on yield. In addition to low yields poor cured leaf quality of this low nicotine variety would make the production of low nicotine tobacco under our present marketing system economically unfeasible for U.S. flue-cured tobacco growers.

The flue curing process and the resulting chemical and physical quality of the cured leaf is largely dependent on the ripeness of the harvested leaf. Excessive use of nitrogen can lead to harvested leaf that is not sufficiently ripe to achieve high quality cured leaf. Low nicotine LA FC53 has an inherently low cured leaf quality and this is likely due to the altered N uptake and utilization. Over fertilization with nitrogen typically results in a lack of ripeness. Low nicotine LA FC53 is not an approved commercial variety and may have atypical N utilization and other features that impact leaf ripeness and subsequent curing and cured leaf quality. More research needs to be conducted to quantify the nitrogen-use efficiency of low nicotine flue-cured tobacco varieties in order to obtain substantially lower nicotine and acceptable cured leaf quality.

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Table 3.1. Analysis of variance for nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and four plant populations combined over both growing seasons (Southern Piedmont AREC).

Effect	Nicotine		Reducing Sugars	
	F value	*P* value	*F* value	*P* value
Year	772.1143	<0.0001	729.7754	<0.0001
Variety	12815.7700	<0.0001	222.1171	<0.0001
N rate	4.4413	0.0473	72.0479	<0.0001
Plant Population	0.3474	0.5619	0.1072	0.7466
Year X Variety	237.7138	<0.0001	3.5307	0.0742
Year X N rate	1.1217	0.3016	1.6508	0.2128
Year X Plant Population	0.3678	0.5507	2.3487	0.1403
Variety X N rate	0.0370	0.8494	0.0436	0.8366
Variety X Plant Population	0.0243	0.8777	0.1281	0.7240
N rate X Plant Population	0.0532	0.8199	0.0367	0.8499

Table 3.2. Composite whole-plant nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and four plant populations in 2019 and 2020 (Southern Piedmont AREC).

Variety	**N Rate (lbs N/ac)**	**Plant Population (plants/ac)**	**Nicotine (%)**	**Reducing Sugars (%)**
2019				
K326	**42 lbs N**	5,940	4.27 *a*	5.86 *a*
		6,534	3.99 *a*	5.67 *a*
		7,260	4.09 *a*	5.94 *a*
		8,168	4.15 *a*	5.92 *a*
	72 lbs N	5,940	4.29 *a*	4.73 *a*
		6,534	4.21 *a*	4.88 *a*
		7,260	4.18 *a*	4.15 *a*
		8,168	4.20 *a*	4.53 *a*
LA FC53	**42 lbs N**	5,940	0.66 *a*	3.19 *a*
		6,534	0.69 *a*	3.40 *a*
		7,260	0.72 *a*	3.98 *a*
		8,168	0.71 *a*	3.56 *a*
	72 lbs N	5,940	0.73 *a*	1.37 *a*
		6,534	0.76 *a*	1.89 *a*
		7,260	0.76 *a*	2.21 *a*
		8,168	0.83 *a*	2.14 *a*
2020				
K326	**42 lbs N**	5,940	2.95 *a*	9.72 *a*
		6,534	3.00 *a*	9.16 *a*
		7,260	3.10 *a*	9.58 *a*
		8,168	2.97 *a*	9.93 *a*
	72 lbs N	5,940	2.94 *a*	7.84 *a*
		6,534	3.04 *a*	9.06 *a*
		7,260	2.96 *a*	8.14 *a*
		8,168	3.19 *a*	8.11 *a*
LA FC53	**42 lbs N**	5,940	0.37 *a*	7.97 *a*
		6,534	0.39 *a*	7.88 *a*
		7,260	0.40 *a*	7.16 *a*
		8,168	0.39 *a*	6.69 *a*
	72 lbs N	5,940	0.41 *a*	6.69 *a*
		6,534	0.49 *a*	6.47 *a*
		7,260	0.43 *a*	7.05 *a*
		8,168	0.35 *a*	6.15 *a*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 3.3. Analysis of variance for yield and grade index of K 326 and LA FC53 grown at two nitrogen rates and four plant populations combined over both growing seasons (Southern Piedmont AREC).

Effect	Yield		Grade Index	
	F value	*P* value	*F* value	*P* value
Year	319.3064	<0.0001	169.3427	<0.0001
Variety	660.7916	<0.0001	1477.3470	<0.0001
N rate	23.8371	<0.0001	12.4848	0.0020
Plant Population	42.8425	<0.0001	3.4244	0.0784
Year X Variety	0.3941	0.5369	0.1188	0.7337
Year X N rate	2.3024	0.1441	0.0668	0.7985
Year X Plant Population	3.5957	0.0718	0.9788	0.3338
Variety X N rate	0.0035	0.9531	3.5947	0.0718
Variety X Plant Population	0.1748	0.6802	0.2532	0.6201
N rate X Plant Population	1.2430	0.2775	5.1507	0.0339

Table 3.4. Yield and grade index of K 326 and LA FC53 grown at two nitrogen rates and four plant populations in 2019 and 2020 (Southern Piedmont AREC).

Variety	N Rate (lbs N/ac)	Plant Population (plants/ac)	Yield (lbs/ac)	Grade Index (0-100)
2019				
K326	**42 lbs N**	5,940	3020 *b*	77 *a*
		6,534	3275 *ab*	75 *a*
		7,260	3041 *b*	81 *a*
		8,168	3395 *a*	79 *a*
	72 lbs N	5,940	3290 *a*	71 *b*
		6,534	3394 *a*	73 *ab*
		7,260	3482 *a*	70 *b*
		8,168	3476 *a*	80 *a*
LA FC53	**42 lbs N**	5,940	2379 *a*	22 *a*
		6,534	2369 *a*	24 *a*
		7,260	2501 *a*	22 *a*
		8,168	2464 *a*	20 *a*
	72 lbs N	5,940	2429 *b*	13 *b*
		6,534	2617 *ab*	15 *b*
		7,260	2709 *a*	12 *b*
		8,168	2646 *a*	28 *a*
2020				
K326	**42 lbs N**	5,940	3665 *b*	64 *a*
		6,534	3698 *b*	58 *a*
		7,260	3918 *a*	70 *a*
		8,168	4051 *a*	56 *a*
	72 lbs N	5,940	3781 *b*	46 *a*
		6,534	3804 *ab*	48 *a*
		7,260	4044 *a*	52 *a*
		8,168	4027 *a*	57 *a*
LA FC53	**42 lbs N**	5,940	2706 *b*	1 *a*
		6,534	2901 *b*	1 *a*
		7,260	3181 *a*	1 *a*
		8,168	3162 *a*	1 *a*
	72 lbs N	5,940	3028 *ab*	1 *a*
		6,534	3018 *b*	2 *a*
		7,260	3199 *ab*	1 *a*
		8,168	3222 *a*	1 *a*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 3.5. Analysis of variance for nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments over both growing seasons (Southern Piedmont AREC).

Effect	Nicotine		Reducing Sugars	
	F value	*P* value	*F* value	*P* value
Year	8.4641	0.0084	70.9082	<0.0001
Variety	132.1351	<0.0001	72.8175	<0.0001
N rate	1.4944	0.2351	34.1540	<0.0001
Topping Time	2.5617	0.1244	23.4616	<0.0001
Year X Variety	0.1146	0.7383	0.0844	0.7743
Year X N rate	0.9477	0.3414	1.8668	0.1863
Year X Topping Time	1.2381	0.2784	4.8862	0.0383
Variety X N rate	1.5188	0.2314	0.3757	0.5465
Variety X Topping Time	0.0515	0.8227	1.2771	0.2712
N rate X Topping Time	0.1640	0.6896	0.3948	0.5366

Table 3.6. Composite whole-plant nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments in 2019 and 2020 (Southern Piedmont AREC).

Variety	N Rate (lbs N/ac)	Topping Time	Nicotine (%)		Reducing Sugars (%)	
2019						
K326	**42 lbs N**	25% Bloom	4.06	*a*	6.61	*a*
		100% Bloom	3.87	*a*	6.27	*a*
		1st Harvest	3.95	*a*	6.33	*a*
		No Topping	3.07	*b*	5.81	*a*
	72 lbs N	25% Bloom	3.84	*ab*	4.73	*a*
		100% Bloom	4.12	*a*	4.97	*a*
		1st Harvest	3.47	*ab*	5.74	*a*
		No Topping	3.05	*b*	4.64	*a*
LA FC53	**42 lbs N**	25% Bloom	0.70	*a*	3.15	*b*
		100% Bloom	0.81	*a*	4.00	*b*
		1st Harvest	0.68	*a*	5.44	*a*
		No Topping	0.25	*b*	3.54	*b*
	72 lbs N	25% Bloom	1.24	*a*	1.95	*a*
		100% Bloom	0.63	*a*	3.06	*a*
		1st Harvest	1.34	*a*	3.32	*a*
		No Topping	0.34	*a*	3.33	*a*
2020						
K326	**42 lbs N**	25% Bloom	3.39	*a*	7.76	*b*
		100% Bloom	3.11	*b*	9.32	*ab*
		1st Harvest	2.95	*b*	10.33	*a*
		No Topping	2.62	*c*	8.61	*ab*
	72 lbs N	25% Bloom	3.44	*a*	7.30	*a*
		100% Bloom	3.32	*a*	6.85	*a*
		1st Harvest	3.06	*ab*	7.68	*a*
		No Topping	2.82	*b*	5.56	*a*
LA FC53	**42 lbs N**	25% Bloom	0.48	*a*	4.56	*b*
		100% Bloom	0.45	*a*	6.36	*b*
		1st Harvest	0.46	*a*	8.95	*a*
		No Topping	0.42	*a*	5.82	*ab*
	72 lbs N	25% Bloom	0.45	*ab*	4.01	*b*
		100% Bloom	0.43	*ab*	4.25	*b*
		1st Harvest	0.50	*a*	6.96	*a*
		No Topping	0.33	*b*	3.95	*b*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 3.7. Analysis of variance for yield and grade index of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments over both growing seasons (Southern Piedmont AREC).

Effect	Yield		Grade Index	
	F value	*P* value	*F* value	*P* value
Year	28.8051	<0.0001	31.2170	<0.0001
Variety	26.5102	<0.0001	757.9445	<0.0001
N rate	0.6218	0.4392	12.2701	0.0021
Topping Time	7.3489	0.0131	0.8932	0.3554
Year X Variety	0.0197	0.8898	1.4088	0.2485
Year X N rate	0.0808	0.7790	7.8853	0.0105
Year X Topping Time	0.0799	0.7803	7.4623	0.0125
Variety X N rate	0.0198	0.8893	5.4985	0.0289
Variety X Topping Time	0.0048	0.9455	3.8217	0.0640
N rate X Topping Time	0.2285	0.6375	1.5127	0.2323

Table 3.8. Yield and grade index of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments in 2019 and 2020 (Southern Piedmont AREC).

Variety	N Rate (lbs N/ac)	Topping Time	Yield (lbs/ac)	Grade Index (0-100)
2019				
K326	**42 lbs N**	25% Bloom	3006 *a*	74 *a*
		100% Bloom	2932 *a*	76 *a*
		1st Harvest	2496 *b*	73 *a*
		No Topping	2037 *c*	70 *a*
	72 lbs N	25% Bloom	3237 *a*	73 *ab*
		100% Bloom	3068 *a*	77 *a*
		1st Harvest	2844 *a*	75 *ab*
		No Topping	2119 *b*	69 *b*
LA FC53	**42 lbs N**	25% Bloom	2327 *a*	1 *b*
		100% Bloom	2137 *a*	22 *a*
		1st Harvest	1791 *b*	27 *a*
		No Topping	1290 *c*	2 *b*
	72 lbs N	25% Bloom	2416 *a*	10 *a*
		100% Bloom	2334 *a*	11 *a*
		1st Harvest	1929 *b*	11 *a*
		No Topping	1323 *c*	7 *a*
2020				
K326	**42 lbs N**	25% Bloom	3669 *b*	75 *a*
		100% Bloom	3793 *a*	76 *a*
		1st Harvest	3378 *c*	63 *a*
		No Topping	2943 *d*	72 *a*
	72 lbs N	25% Bloom	3923 *a*	49 *a*
		100% Bloom	3677 *ab*	36 *a*
		1st Harvest	3640 *b*	44 *a*
		No Topping	2833 *c*	55 *a*
LA FC53	**42 lbs N**	25% Bloom	2833 *b*	3 *a*
		100% Bloom	3061 *a*	6 *a*
		1st Harvest	2738 *b*	1 *a*
		No Topping	2215 *c*	1 *a*
	72 lbs N	25% Bloom	3021 *a*	1 *a*
		100% Bloom	3202 *a*	1 *a*
		1st Harvest	2837 *a*	1 *a*
		No Topping	2087 *b*	1 *a*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD ($\alpha = 0.05$).

Table 3.9. Analysis of variance for nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and three topping heights over both growing seasons (Southern Piedmont AREC).

Effect	Nicotine		Reducing Sugars	
	F value	*P* value	*F* value	*P* value
Year	43.6382	<0.0001	37.0548	<0.0001
Variety	6256.9170	<0.0001	135.4683	<0.0001
N rate	2.2210	0.1600	5.5381	0.0350
Topping Height	33.7287	<0.0001	7.3549	0.0178
Year X Variety	12.2793	0.0039	1.0738	0.3190
Year X N rate	2.5380	0.1352	0.2381	0.6337
Year X Topping Height	53.0798	<0.0001	3.5071	0.0838
Variety X N rate	2.1037	0.1706	0.9418	0.3495
Variety X Topping Height	0.2256	0.6427	0.0005	0.9817
N rate X Topping Height	0.2051	0.6581	0.4997	0.4921

Table 3.10. Composite whole-plant nicotine and reducing sugars of K 326 and LA FC53 grown at two nitrogen rates and three topping heights in 2019 and 2020 (Southern Piedmont AREC).

Variety	N Rate (lbs N/ac)	Topping Time	Nicotine (%)		Reducing Sugars (%)	
2019						
K326	**42 lbs N**	16 leaves	3.87	*a*	5.51	*a*
		20 leaves	3.63	*a*	6.00	*a*
		24 leaves	4.24	*a*	5.65	*a*
	72 lbs N	16 leaves	3.56	*a*	5.03	*a*
		20 leaves	3.57	*a*	5.85	*a*
		24 leaves	3.95	*a*	5.58	*a*
LA FC53	**42 lbs N**	16 leaves	0.74	*a*	3.80	*a*
		20 leaves	0.72	*a*	3.53	*a*
		24 leaves	0.75	*a*	3.91	*a*
	72 lbs N	16 leaves	0.67	*a*	3.33	*a*
		20 leaves	0.71	*a*	3.01	*a*
		24 leaves	0.78	*a*	3.35	*a*
2020						
K326	**42 lbs N**	16 leaves	3.38	*a*	7.19	*a*
		20 leaves	3.42	*a*	7.39	*a*
		24 leaves	3.30	*a*	8.15	*a*
	72 lbs N	16 leaves	3.47	*a*	5.65	*a*
		20 leaves	3.42	*a*	7.16	*a*
		24 leaves	3.22	*a*	6.63	*a*
LA FC53	**42 lbs N**	16 leaves	0.41	*a*	3.93	*a*
		20 leaves	0.50	*a*	4.93	*a*
		24 leaves	0.41	*a*	4.69	*a*
	72 lbs N	16 leaves	0.41	*a*	3.24	*b*
		20 leaves	0.47	*a*	5.16	*a*
		24 leaves	0.45	*a*	5.03	*a*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 3.11. Analysis of variance for yield and grade index of K 326 and LA FC53 grown at two nitrogen rates and three topping heights over both growing seasons (Southern Piedmont AREC).

Effect	Yield		Grade Index	
	F value	*P* value	*F* value	*P* value
Year	231.9304	<0.0001	103.7140	<0.0001
Variety	46.2228	<0.0001	1689.5480	<0.0001
N rate	4.4112	0.0558	0.5420	0.4747
Topping Height	19.4608	0.0007	9.0330	0.0101
Year X Variety	35.1072	<0.0001	33.2565	<0.0001
Year X N rate	0.1279	0.7264	0.9635	0.3442
Year X Topping Height	0.5134	0.4863	8.1523	0.0135
Variety X N rate	3.3193	0.0915	1.5055	0.2416
Variety X Topping Height	1.0264	0.3295	1.1065	0.3120
N rate X Topping Height	0.0281	0.8694	2.7325	0.1223

Table 3.12. Yield (a) and grade index (b) of K 326 and LA FC53 grown at two nitrogen rates and three topping heights in 2019 and 2020 (Southern Piedmont AREC).

Variety	N Rate (lbs N/ac)	Topping Time	Yield (lbs/ac)		Grade Index (0-100)	
2019						
K326	**42 lbs N**	16 leaves	2511	*c*	74	*a*
		20 leaves	2807	*b*	73	*a*
		24 leaves	3139	*a*	74	*a*
	72 lbs N	16 leaves	2634	*b*	60	*a*
		20 leaves	2919	*ab*	73	*a*
		24 leaves	3144	*a*	72	*a*
LA FC53	**42 lbs N**	16 leaves	2083	*a*	1	*a*
		20 leaves	2135	*a*	4	*a*
		24 leaves	2106	*a*	16	*a*
	72 lbs N	16 leaves	2046	*b*	1	*a*
		20 leaves	2350	*a*	9	*a*
		24 leaves	2259	*a*	13	*a*
2020						
K326	**42 lbs N**	16 leaves	3462	*b*	51	*a*
		20 leaves	3711	*a*	52	*a*
		24 leaves	3782	*a*	45	*a*
	72 lbs N	16 leaves	3452	*b*	45	*a*
		20 leaves	4049	*a*	51	*a*
		24 leaves	3780	*ab*	52	*a*
LA FC53	**42 lbs N**	16 leaves	2800	*b*	1	*a*
		20 leaves	3006	*a*	1	*a*
		24 leaves	3064	*a*	1	*a*
	72 lbs N	16 leaves	2806	*b*	1	*a*
		20 leaves	3109	*a*	3	*a*
		24 leaves	3104	*a*	1	*a*

Means followed by the same letter within a column grouped by variety and N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

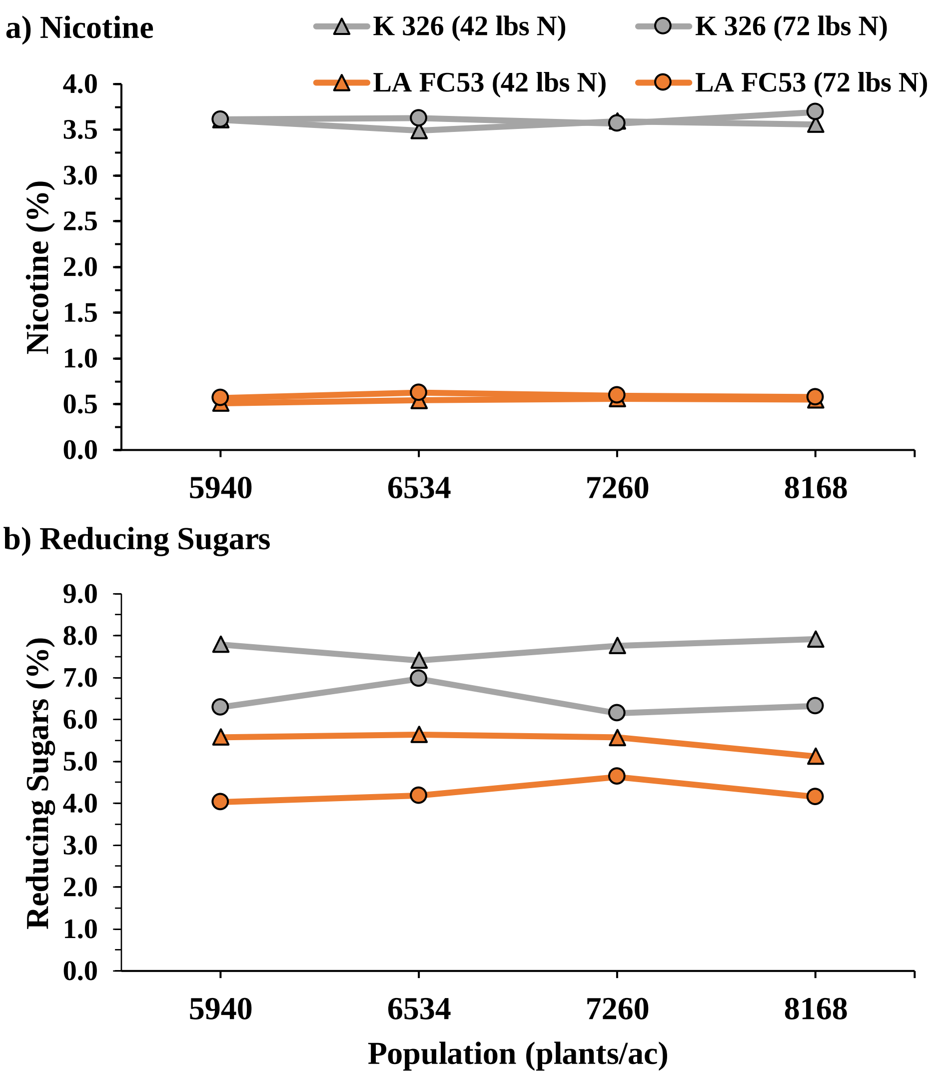
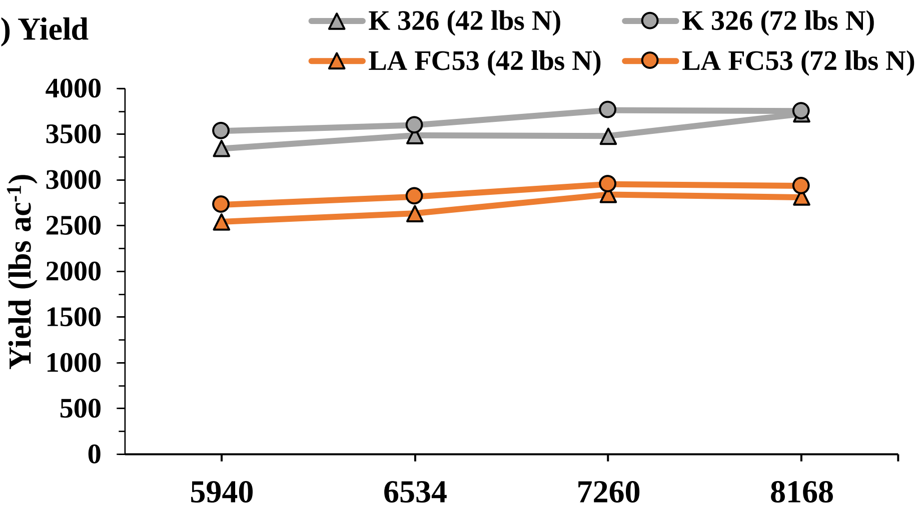


Figure 3.1 a-b. Composite whole-plant nicotine (a) and reducing sugars (b) of K 326 and LA FC53 grown at two nitrogen rates and four plant populations. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

a) Yield



b) Grade Index

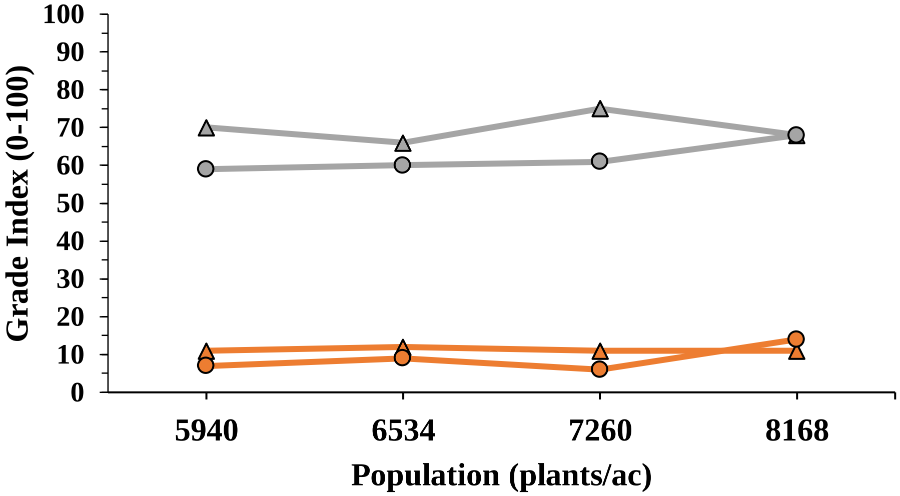
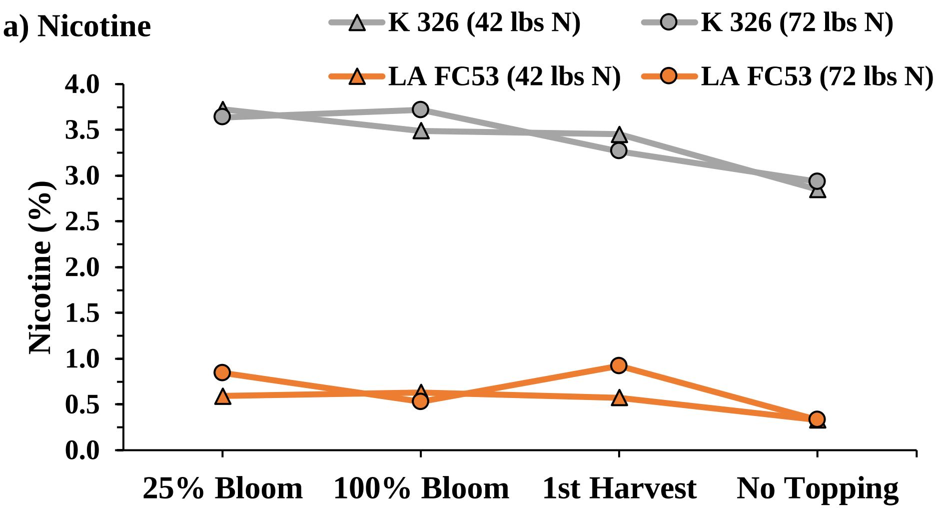


Figure 3.2 a-b. Yield (a) and grade index (b) of K 326 and LA FC53 grown at two nitrogen rates and four plant populations. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

a) Nicotine



b) Reducing Sugars

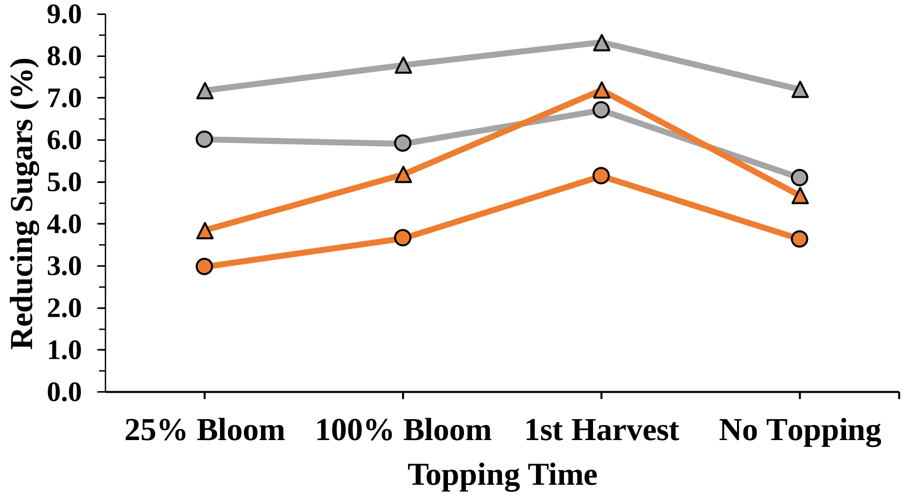


Figure 3.3 a-b. Composite whole-plant nicotine (a) and reducing sugars (b) of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

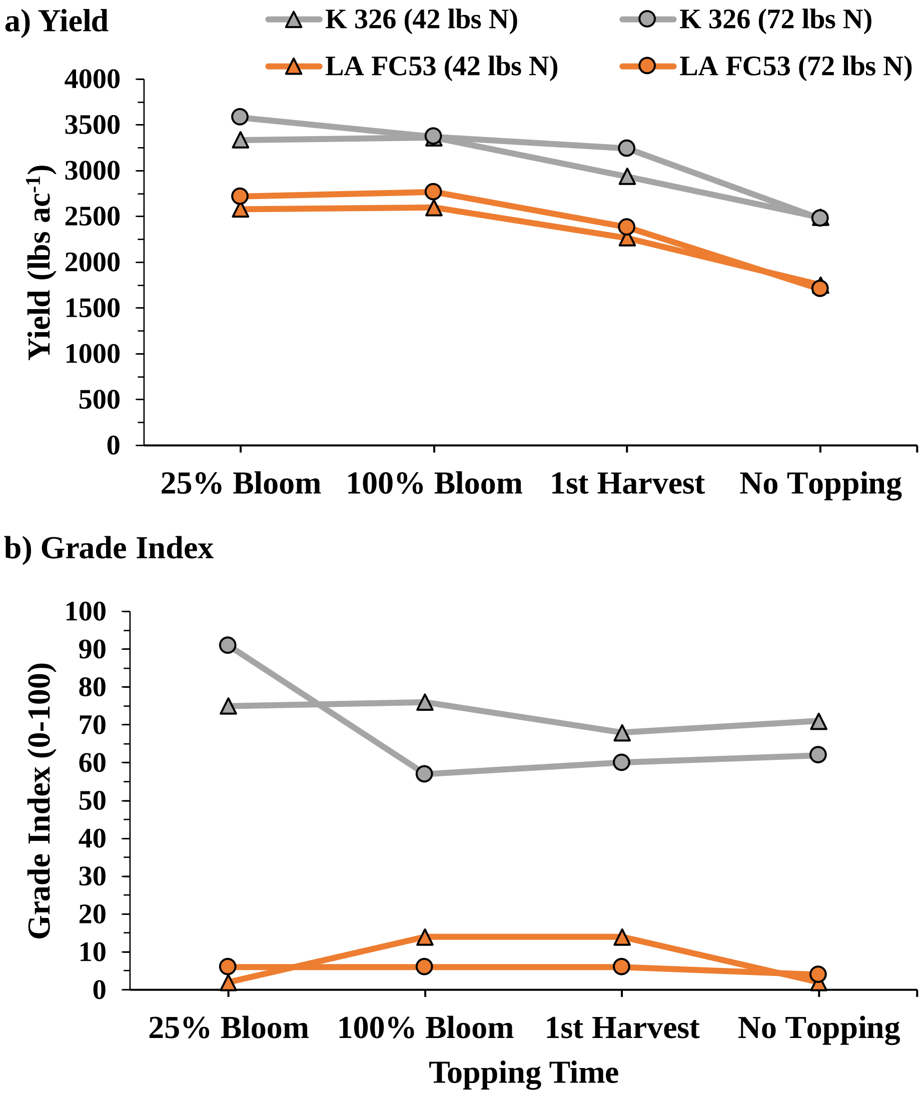
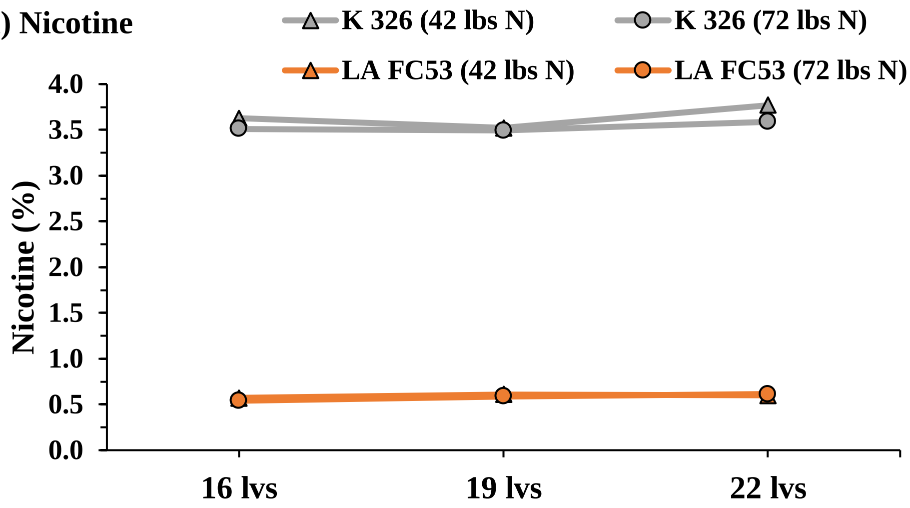


Figure 3.4 a-b. Yield (a) and grade index (b) of K 326 and LA FC53 grown at two nitrogen rates and four topping time treatments. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

a) Nicotine



b) Reducing Sugars

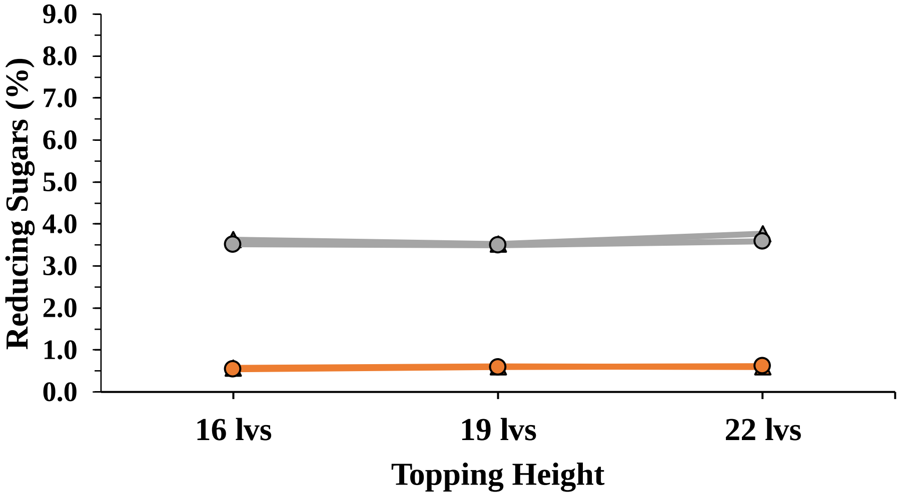
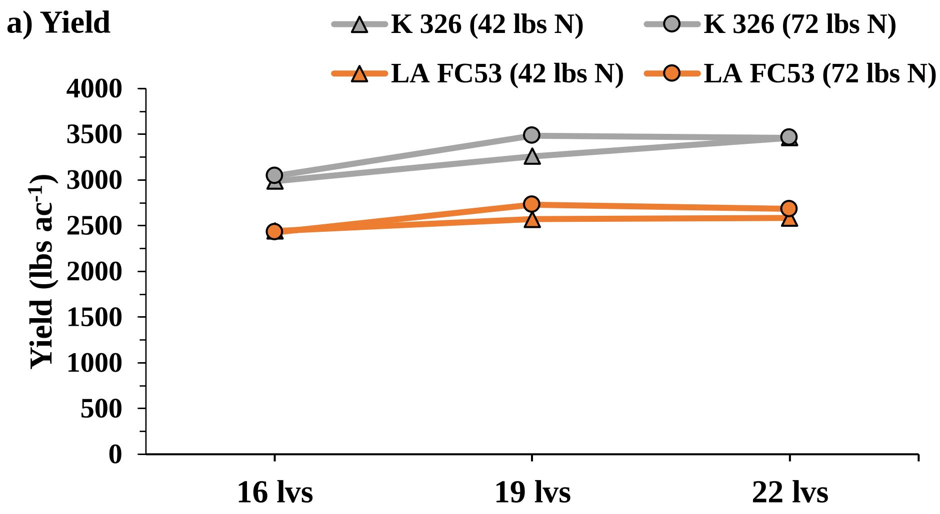


Figure 3.5 a-b. Composite whole-plant nicotine (a) and reducing sugars (b) of K 326 and LA FC53 grown at two nitrogen rates and three topping heights. Data are combined over 2019 and 2020 (Southern Piedmont AREC).



a) Yield

b) Grade Index

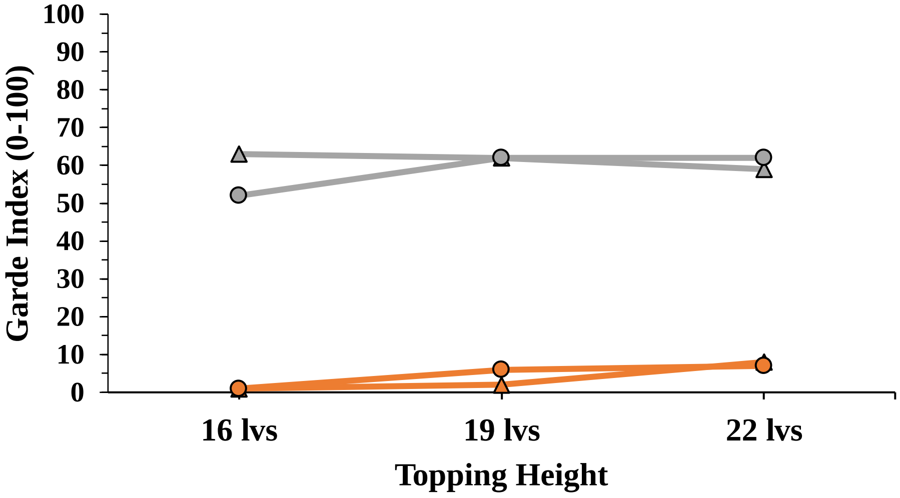


Figure 3.6 a-b. Yield (a) and grade index (b) of K 326 and LA FC53 grown at two nitrogen rates and three topping heights. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

Chapter IV

Nitrogen-Use Efficiency of Low-Nicotine Flue-Cured Tobacco Varieties

Introduction

Flue-cured tobacco yields have trended upward for over the past 100 years due to advancements in cultivars, production practices, and technology (Sisson *et al*., 1991). A large improvement to the commercial production of tobacco was the implementation of increased nitrogen fertilizer rates. The most important macronutrient in flue-cured tobacco production is nitrogen and adequate levels are required to produce high-yielding, high quality leaf *(*Drake *et al*., 2015). With increased rates of nitrogen fertilizers and the development of new varieties inherently came an overall improvement in nitrogen-use efficiency (Sisson *et al*., 1991).

Research has been conducted to better develop flue-cured tobacco varieties that produce less nicotine due to potential mandates to drastically lower nicotine levels in cigarette products (FDA, 2018; Murillo, 2018). The goal is to produce varieties that are lower in nicotine but maintain adequate yield and cured leaf quality. Nitrogen has been shown to be the most influential factor in yield, quality, and nicotine production (Collins & Hawks, 1983). Understanding nitrogen-use efficiency and the parameters thereof is important to better develop varieties that can reach the FDA proposed nicotine levels of 0.3-0.5 mg nicotine per g of tobacco (Murillo, 2018) while still providing proper yield and quality. The objective of the research described herein was to quantify differences in nitrogen-use efficiency between conventional flue-cured tobacco varieties and low nicotine alternative varieties.

Materials and Methods

A study was conducted to evaluate the nitrogen-use efficiency of recently developed, low-nicotine varieties of flue-cured tobacco at the Southern Piedmont Agricultural Research and Extension Center near Blackstone, VA during the 2019 and 2020 growing seasons. Rates of nitrogen fertilizer were decreased from the standard production rate of 72 lbs N/ac with the intent of testing the efficiency of five individual varieties at lower rates of nitrogen.

Tobacco was planted on raised beds spaced 48 in. apart and plots were 40 ft. long with an in-row plant spacing of 22 in. Individual plots consisted of two rows, a row for in-season plant sampling and a harvest row. All plots were fertilized at formation of the beds with 700 lbs/ac of

0-6-18 tobacco grade fertilizer. The rates of N tested were 29, 43, 58, and 72 lbs N/ac using UAN-30 at 8.9, 13.3, 17.9, and 22.2 gal/ac, respectively. The liquid N solution was applied using a two-row JohnBlue squeeze pump injection rig (JohnBlue Company, Huntsville, AL) the day following transplanting. All other agronomic practices followed recommendations from the 2019 Flue-Cured Tobacco Production Guide (Reed *et al.*, 2018).

The N fertilization rate, cured leaf yield, and total nitrogen (TN) levels of a composite whole-plant sample of the cured leaf of three low-nicotine alternative varieties (LA FC53, Altria1, and Altria2) and two conventional varieties (NC 95 and K 326) were used to determine their nitrogen-use efficiency. The calculation methods have been modeled and altered by two previous nitrogen efficiency projects done on corn (*Zea mays* L.) and flue-cured tobacco (*Nicotiana tabacum*) (Moll *et al*., 1982; Sisson *et al*., 1991). Modifications done by Sisson (1991) were to focus calculations on cured leaf yield instead of seed yield. These appropriate changes were needed due to calculations done by Moll (1982) that were for grain yield of corn. Nitrogen-use efficiency was determined based on calculations of the following parameters:

Nitrogen-Use (N-USE) = yield (lbs/ac)/units N fertilizer (lbs N/ac)

Nitrogen-Uptake (N-UPT) = N-ACC (lbs/ac)/units N fertilizer (lbs N/ac)

Nitrogen-Utilization (N-UTL) = yield (lbs/ac)/N-ACC (lbs/ac)

Nitrogen-Accumulation (N-ACC) = yield (lbs/ac) × TN concentration (ppm)

Tobacco was harvested four times as leaves ripened, beginning at the bottom of the stalk and progressing up the stalk. Yield was calculated as the sum of the individual harvest weights. In-season plant N status was monitored by measuring nitrate levels in petiole sap samples. Samples for petiole nitrate monitoring were taken throughout the growing season from the third leaf from the top of the plant at layby, topping, and throughout the four harvests. Petiole nitrate samples were also taken at each stalk position throughout all four harvests. The basal position (6 in.) of the midrib was pressed using a hydraulic laboratory press. Petiole sap was analyzed for nitrates using a LAQUA Twin Nitrate NO_3^- Meter (HORIBA Instruments Incorporated, Irvine, CA). Specific leaf weight samples were collected at the fourth leaf from the top just prior to final harvest to quantify potential differences in leaf body and texture. Leaf disks were cut from the leaf lamina in the middle third of the leaf on either side of the midrib then dried before being weighed. Four stalks and their attached root systems were collected from each plot after the final harvest. These samples were air dried and weighed separately for an average root weight and stalk weight for each plot. All cured leaf was graded by a USDA AMS flue-cured tobacco grader and corresponding grade index value assigned according to Bowman et al. (1988). An overall or composite grade index value was calculated for each plot based on the proportion of the total yield represented by each harvest and their corresponding yield weights. Composite nicotine, reducing sugars, and total N were calculated in a similar fashion. Nicotine and reducing sugar levels were determined using a FOSS-5000® NIR (FOSS Analytical, Eden Prairie, MN). A vario EL cube CHNS elemental analyzer was used to analyze cured leaf samples for TN (Elementar Americas Inc., Ronkonkoma, NY). Data only uses TN determined from leaf laminae so these values only refer to cured leaf TN.

The study was arranged as a split-plot design with N rate as the whole-plot variable and variety as the split-plot variable. Treatments were replicated four times in each field. Analysis of variance was run using SAS 9.4 (SAS Institute, 2019) and significant differences determined using Fisher's protected LSD ($\alpha = 0.05$).

Results and Discussion

Low-Nicotine & Conventional Flue-Cured Tobacco Varieties

Petiole Nitrate Monitoring

Leaf petiole sap nitrate levels of the third leaf from the top of the plant are shown in figures 4.1 a-d. Petiole sap nitrate levels declined more from layby to topping as the N rate was decreased from the standard. Low nicotine varieties showed a consistently higher level of petiole nitrates sampled at the top of the plant compared to the conventional varieties. At layby, low nicotine varieties had the highest and lowest levels of petiole nitrates at each N rate. This trend was similar in samples taken at topping except in 58 lbs N/ac where K 326 had the lowest level of nitrates. Samples taken from the top of the plant tended to be highest in nitrate in low-nicotine varieties at each harvest date. Nitrate samples taken from these same harvest dates tended to be lower in the conventional varieties except T1 at 29 lbs N/ac where Altria1 was the lowest. Averaged over all N rates, low-nicotine varieties were consistently higher in petiole nitrates than the conventional varieties.

Nitrate levels monitored in leaves from each harvest were lowest in NC 95 except the lug (X) position at 29 lbs N/ac where LA FC53 was lowest (Figs. 4.2 a-d). Nitrate levels at the tip (T) stalk position were highest in low-nicotine varieties and lowest in the conventional at all N rates. Averaged over all N rates, NC 95 tended to be the lowest in petiole sap nitrate levels. Petiole sap nitrate levels in harvested leaves decreased with progressive harvests of the leaves from the bottom (X or lugs) to the top of the stalk (T or tips).

Total Nitrogen

Effects from year, variety, N rate, and an interaction of year X N rate ($P < 0.0001$) was highly significant for level of TN (Table 4.1) Levels of TN in 2019 were significantly higher in the conventional varieties at each N rate except 58 lbs N/ac (Table 4.2). At 58 lbs N/ac, only NC 95 and Altria2 showed a significant difference from one another. During 2020, the conventional varieties remained the highest at 43 lbs N/ac and was no different from LA FC53 at 29 and 72 lbs N/ac. At 58 lbs N/ac, Altria1 and Altria2 were not significantly different from the conventional varieties.

Total nitrogen averaged from both growing seasons showed the two conventional varieties to have the highest TN at each N rate compared to the low-nicotine varieties (Fig. 4.3). Of the low-nicotine varieties tested, LA FC53 was the highest in TN at every N rate except 58 lbs N/ac which was highest in Altria1 and Altria2. As expected, each variety tended to decrease in TN levels as N rate decreased from the standard.

Nitrogen-Use

Nitrogen-use measures the capacity of a variety to maximize the N applied by providing the pounds of cured leaf produced by a single pound of applied N. Effects from year, variety, N rate, and an interaction of year X N rate were significant to the N-USE of these varieties (Table 4.1). In 2019, N-USE was highest in K 326, Altria1, and Altria2 at each N rate except the standard (Table 4.2). At a standard N rate of 72 lbs N/ac, Altria2 was not significantly different from the lowest varieties NC 95 and LA FC53. Varieties NC 95 and LA FC53 were consistently the lowest in N-USE and in most cases significantly lower. In 2020, K 326 was significantly higher in N-USE at 29 and 58 lbs N/ac compared to the other varieties tested. At 43 lbs N/ac, K 326 and Altria1 were the highest with NC 95, LA FC53, and Altria2 being significantly lower. Varieties NC 95 and K 326 were the highest in N-USE at 72 lbs N/ac and LA FC53 was significantly lower. The lowest N-USE values were found in LA FC53 at each N rate during 2019 and 2020.

Averaged over both years, K 326 exhibited the highest N-USE levels and LA FC53 had the lowest of any variety at each N rate (Fig. 4.4). Varieties Altria1 and Altria2 were lower in N-USE than K 326 but still higher than NC 95 and LA FC53. These updated varieties were the top performing low-nicotine varieties tested for N-USE. A trend of increasing N-USE as the N rate was lowered can be seen with each variety.

Nitrogen-Uptake

Nitrogen-uptake (N-UPT) measures the ability of a variety to absorb N by comparing the rate of N applied to the amount of TN found in the plant. Effects from year, variety, N rate, and an interaction of year X N rate were highly significant to N-UPT ($p < 0.0001$) (Table 4.1). During 2019, K 326 was significantly higher in N-UPT than all other varieties tested at 29 and 72 lbs N/ac and there was greater separation among the varieties at the intermediate levels of N (Table 4.2). At the lowest and standard N rate, no significant differences were found between the low-nicotine varieties and NC 95 and were all the lowest in N-UPT. Variety LA FC53 was the lowest in N-UPT at 43 and 58 lbs N/ac but not significantly different from Altria1 and Altria2 at 58 lbs N/ac. Of the low-nicotine varieties tested, Altria1 and Altria2 tended to be the highest in N-UPT. Nitrogen-uptake was significantly higher in K 326 and NC 95 at 29 and 43 lbs N/ac during 2020. At 58 lbs N/ac, K 326 was significantly higher than all varieties including NC 95 but was not different at 72 lbs N/ac. All low-nicotine varieties were significantly lower than the conventional varieties at 29 and 43 lbs N/ac but Altria1 and Altria2 tended to be the highest of the low-nicotine varieties and statistically higher at 43 lbs N/ac. Variety LA FC53 was, in most cases significantly, the lowest in N-UPT at each N rate during 2019 and 2020.

Averaged over both years, the conventional varieties were the highest in N-UPT at each N rate (Fig. 4.5). Of the low-nicotine varieties tested, Altria1 and Altria2 were the highest in N-UPT at each N rate. The lowest N-UPT at each N rate was consistently found in LA FC53 with levels as much as 1/3 lower than K 326.

Nitrogen-Utilization

Nitrogen-utilization (N-UTL) measures how well the plant has utilized N by comparing its yield to N accumulated in the cured leaf. Effects from year, variety, N rate, and an interaction of year X N rate were highly significant to N-UTL ($p < 0.0001$) (Table 4.1). An interaction of year X variety also had a significant impact on N-UTL ($p = 0.0441$). In the 2019 growing season, each low-nicotine variety was significantly higher in N-UTL at the standard N rate (Table 4.2). Varieties Altria1 and Altria2 were the highest in N-UTL at 29 lbs N/ac. All of the low-nicotine varieties were significantly higher than NC 95 but no different from K 326 at 43 lbs N/ac. At 58 lbs N/ac, Altria2 was the highest in N-UTL but no different from LA FC53, K 326, of Altria1. At the lowest N rate in 2020, Altria1 and Altria2 were significantly higher than the other varieties in N-UTL. All of the low-nicotine varieties were higher in N-UTL at the two intermediate N rates. Varieties Altria1 and Altria2 were the highest in N-UTL at a standard N rate but NC 95 and LA FC53 were not statistically different. A consistent trend over both years is low-nicotine varieties tend to be higher in N-UTL than the conventional varieties.

Levels of N-UTL averaged over both years shows Altria1 and Altria2 to be the highest in N-UTL at every N rate except 58 lbs N/ac where LA FC53 was the highest (Fig. 4.6). Both conventional varieties were the lowest in N-UTL at each N rate. This trend is due mainly to the N-ACC levels being higher in conventional varieties and lower in low-nicotine varieties. In all varieties, N-UTL trended upward as N rate was lowered from the standard rate.

Nitrogen Accumulation

Nitrogen accumulation (N-ACC) describes the amount of TN found in the cured leaf. Effects from year, variety, and N rate had a highly significant effect on N-ACC ($p < 0.0001$) (Table 4.1) Interactions from year X N rate and year X variety were also highly significant to N-ACC ($p < 0.0001$ and $p = 0.0137$, respectively). Results from 2019 showed K 326 had the highest levels of N-ACC at 72 lbs N/ac (Table 4.2). Variety LA FC53 was significantly lower than each variety at 43 lbs N/ac. At 58 lbs N/ac, LA FC53 and Altria1 were significantly lower than K 326. Variety NC 95 was not different from K 326 at the lowest N rate and LA FC53, Altria1, and Altria2 were significantly lower. At each N rate tested, LA FC53 was the lowest of all varieties tested. In 2020, all of the low-nicotine varieties were significantly lower than the conventional varieties at 29 and 43, and 58 lbs N/ac. At a standard N rate, only LA FC53 and Altria1 were significantly lower than K 326. Both NC 95 and Altria2 were not significantly different in than K 326 in N-ACC at a standard N rate.

Data averaged over both growing seasons shows a trend between all varieties of an increase in N-ACC as the applied N rate increases (Fig. 4.7). Both conventional varieties had a higher N-ACC than the low-nicotine varieties at each N rate with K 326 having the highest level. Of the low-nicotine varieties tested, Altria1 and Altria2 were the highest in N-ACC and LA FC53 was the lowest.

Cured Leaf Yield and Quality

Effects from year, variety, N rate, and an interaction of year X N rate were highly significant to yield (Table 4.3). Cured leaf quality, measured as grade index, of the varieties tested were significantly affected by year and variety ($p = 0.0066$ and $p <0.0001$, respectively). Yield from 2019 was significantly lower in NC 95 and LA FC53 at each N rate and Altria2 was no different at the standard rate of 72 lbs N/ac (Table 4.4). Variety K 326 was the highest at each N rate except 43 lbs N/ac where Altria1 was the highest. Statistically, varieties Altria1 and Altria2 tended to be no different in yield than K 326 at each N rate except Altria2 at 72 lbs N/ac. Grade index in 2019 was highest in K 326 at 29 lbs N/ac but no different from NC 95, Altria1, and Altria2 at the intermediate N rates. At the standard 72 lbs N/ac, NC 95, Altria1, and Altria2 were not significantly different from K 326 in grade index. Yield of each variety was higher at each N rate in 2020 than 2019. Variety K 326 was significantly higher at 29 and 58 lbs N/ac. At 43 lbs N/ac, Altria1 was no different from K 326 in yield. Of the low-nicotine varieties, Altria1 and Altria2 were the highest yielding at each N rate. At the lowest and standard N rate in 2020, each variety was significantly higher than LA FC53 in grade index. Grade index was highest in K 326 at 58 lbs N/ac but Altria1 and Altria2 were not statistically different. At 43 lbs N/ac, Altria2 was no different from K 326 in the highest grade index level. Variety LA FC53 was significantly lower in grade index than all other varieties at each N rate. Of the low nicotine varieties tested, Altria2 was consistently the highest in grade index at each N rate from both years but often not too different from Altria1.

Data analyzed over both growing seasons shows the lowest yielding variety to consistently be LA FC53 at each N rate (Fig. 4.8a). Of the low-nicotine varieties tested, Altria1 and Altria2 were the highest yielding and consistently higher than NC 95 but lower than K 326.

Variety K 326 had the highest grade index of any variety at each N rate (Fig. 4.8b). Of the low nicotine varieties tested, Altria2 was the highest in grade index at each N rate. Variety Altria2 was consistently higher in grade index than conventional variety NC 95 but still lower than K 326. Grade index of LA FC53 was considerably lower than all of the varieties at each N rate.

Cured Leaf Chemistry

Nicotine levels were significantly impacted by variety, N rate, and an interaction of year X N rate (Table 4.5). Effects from year, variety, N rate, and an interaction of year X N rate were highly significant to levels of reducing sugars ($p < 0.0001$). Trends of nicotine and reducing sugars among varieties were unchanged between years. In 2019, both conventional varieties were significantly higher in nicotine than the low-nicotine varieties at each N rate (Table 4.4). The lowest levels of nicotine were consistently found in Altria1 and Altria2 at each N rate. At the intermediate N rates, LA FC53 was not significantly different from Altria1 and Altria2. Of the low-nicotine varieties tested, LA FC53 tended to have the highest nicotine level at each N rate even though not statistically different. Although close, Altria1 and Altria2 never fell within the proposed range for nicotine levels. Levels of reducing sugars were significantly lower in LA FC53, Altria1, and Altria2 at each N rate lowered from the standard 72 lbs N/ac. At the standard N rate, no varieties were significantly different from one another in reducing sugars. Similar to 2019, nicotine levels were significantly higher in both conventional varieties during 2020. Varieties LA FC53, Altria1, and Altria2 were the lowest in nicotine at each N rate. Variety LA FC53 fell within the proposed nicotine range at the two intermediate N rates while Altria1 and Altria2 consistently fell within this proposed range at each N rate. Levels of reducing sugars were highest for the conventional varieties at each N rate.

Nicotine levels averaged over both years were significantly higher in the conventional varieties, as expected (Fig. 4.9a). Of the low-nicotine varieties tested, LA FC53 was the highest in nicotine at every N rate. The lowest levels of nicotine were found in Altria1 and Altria2 at every N rate and consistently fell within the proposed range of nicotine. Reducing sugars tended to increase with a lowered N rate in all varieties tested (Fig. 4.9b). Varieties NC 95 and K 326 were much higher in reducing sugars and not much difference was found between the low-nicotine varieties.

Specific Leaf, Stalk, and Root Weight

Specific leaf weights averaged over both growing seasons do not seem to vary between varieties at any N rate (Fig. 4.10a). As N rate was lowered from the standard 72 lbs N/ac, varieties tended to increase in specific leaf weight. Stalk weight tended to be lowest in K 326 at each N rate except the lowest (Fig. 4.10b). As N rate was lowered from the standard, all varieties tended to decrease in stalk weight. This trend was very similar to root weight with a decrease in weight as N rate was lowered (Fig. 4.10c). Greater separation in root weight between varieties is seen as N rate increases to the standard.

Conclusions

The amount of rainfall each year seemed to have an effect on the data collected from these experiments. In 2020, rainfall during the flue-cured growing season at the Southern Piedmont AREC (Table 4.7) was almost eight inches greater than that of 2019. With this increase in rainfall also came an increase in yield and overall nitrogen-use efficiency for all of the varieties tested. We believe this increase in nitrogen-use efficiency to be more closely related to the rather large increase in yield from 2019 to 2020. Nicotine levels tended to decrease with more rainfall and this could be due to the lessened need to develop a larger root system. Smaller root systems in tobacco would, in most cases, result in fewer points of nicotine synthesis found in the root tips.

Over the course of this research study, the low-nicotine varieties tested were almost always out performed by K 326 in important areas such as yield, grade index, and N-USE. In those same important areas, proprietary varieties Altria1 and Altria2 tended to always be higher than NC 95. This research was done to determine how well low-nicotine varieties could perform at lowered N rates compared to conventional varieties while falling within the FDA proposed range for nicotine. In data averaged over both years, variety LA FC53 consistently showed the lowest levels of yield, quality, and N-USE of all varieties tested. This variety also only fell within the proposed nicotine range once.

The most successful low-nicotine varieties tested were Altria1 and Altria2, derived from K 326, due to nicotine levels consistently falling within the proposed nicotine range and showing promising levels of yield, quality, and N-USE (Table 4.6). Variety Altria1 was always higher than the conventional variety NC 95 in both yield and N-USE but tended to have a lower grade index. The yield and N-USE of Altria2 did not differ much from Altria1; however, the grade index was always higher. The grade index of Altria2 was, in some cases, anywhere between 11% and 25% higher than Altria1 and always higher than NC 95 which is known for its greener color. This increased grade index level could be attributed to being bred specifically for better quality while Altria1 was not. Besides the quality of Altria1, these two varieties showed competitive results in yield, grade index, and N-USE when compared to conventional variety NC 95. The only publicly available low-nicotine variety, LA FC53, did not perform well enough to meet the needs of the proposed nicotine levels or standards required by the industry. This poor performance by LA FC53 could be attributed to its parent line, NC 95, and tendency to be greener much longer in the field. The results from this study indicate that certain recently developed, low-nicotine varieties have the potential to comply with FDA proposed levels while maintaining important standards. No work was done to evaluate smoking characteristics of cured leaf from these studies; however, more research should be conducted to quantify the smoking quality of these low-nicotine varieties.

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Table 4.1. Analysis of variance for the parameters of nitrogen-use efficiency of two conventional and three low-nicotine varieties grown at four nitrogen rates combined over 2019 and 2020 (Southern Piedmont AREC).

Effect	Total N		N-USE		N-UPT		N-UTL		N-ACC	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Year	375.8133	<0.0001	53.9921	<0.0001	171.9057	<0.0001	390.2874	<0.0001	796.5124	<0.0001
Variety	47.2003	<0.0001	3.4348	0.0147	7.9900	<0.0001	44.3166	<0.0001	46.5167	<0.0001
N rate	99.0378	<0.0001	271.0106	<0.0001	208.2064	<0.0001	134.2622	<0.0001	111.2590	<0.0001
Year X Variety	0.4031	0.8426	0.2685	0.9266	0.6385	0.6721	2.6350	0.0441	3.4862	0.0137
Year X N rate	55.2611	<0.0001	16.4188	0.0003	54.2519	<0.0001	90.9573	<0.0001	48.2251	<0.0001
Variety X N rate	0.2116	0.9548	0.3997	0.8450	0.5285	0.7528	1.4815	0.2263	0.4766	0.7906

Table 4.2. Parameters of nitrogen-use efficiency of two conventional and three low-nicotine varieties grown with four nitrogen rates in 2019 and 2020 (Southern Piedmont AREC).

N Rate (lbs N/ac)	**Variety**	**Total N (%)**	**N-USE (lb/lb)**	**N-UPT (lb/lb)**	**N-UTL (lb/lb)**	**N-ACC (lb/ac)**
			2019			
29 lbs N	NC95	1.99 *a*	72.33 *b*	1.45 *ab*	50.51 *c*	42.19 *ab*
	LAFC53	1.75 *b*	71.23 *b*	1.27 *b*	57.69 *b*	36.77 *b*
	K326	1.98 *a*	89.25 *a*	1.79 *a*	51.35 *c*	51.95 *a*
	Altria1	1.58 *b*	80.27 *ab*	1.27 *b*	63.59 *a*	36.92 *b*
	Altria2	1.65 *b*	80.32 *ab*	1.34 *b*	60.87 *ab*	38.78 *b*
43 lbs N	NC95	2.19 *a*	52.65 *bc*	1.16 *a*	45.88 *b*	50.02 *a*
	LAFC53	1.94 *bc*	44.12 *c*	0.87 *b*	52.07 *a*	37.47 *b*
	K326	2.07 *ab*	62.13 *ab*	1.28 *a*	49.83 *ab*	55.03 *a*
	Altria1	1.84 *c*	62.78 *a*	1.17 *a*	55.15 *a*	50.33 *a*
	Altria2	1.85 *bc*	61.80 *ab*	1.16 *a*	54.64 *a*	49.91 *a*
58 lbs N	NC95	2.36 *a*	42.23 *bc*	1.02 *a*	42.94 *b*	58.88 *ab*
	LAFC53	2.02 *ab*	38.37 *c*	0.78 *b*	49.67 *ab*	45.47 *c*
	K326	2.18 *ab*	52.00 *a*	1.14 *a*	46.02 *ab*	66.31 *a*
	Altria1	2.05 *ab*	46.00 *ab*	0.94 *ab*	49.20 *ab*	54.34 *bc*
	Altria2	1.98 *b*	48.99 *a*	0.97 *ab*	50.54 *a*	56.13 *ab*
72 lbs N	NC95	2.57 *a*	35.24 *c*	0.90 *b*	39.12 *b*	65.06 *b*
	LAFC53	2.20 *b*	36.92 *c*	0.81 *b*	46.13 *a*	58.04 *b*
	K326	2.49 *a*	44.91 *a*	1.13 *a*	40.38 *b*	81.09 *a*
	Altria1	2.09 *b*	42.05 *ab*	0.88 *b*	47.84 *a*	63.49 *b*
	Altria2	2.20 *b*	39.77 *bc*	0.88 *b*	45.56 *a*	63.30 *b*
			2020			
29 lbs N	NC95	2.60 *a*	114.32 *b*	3.00 *a*	39.35 *c*	86.90 *a*
	LAFC53	2.45 *ab*	97.93 *c*	2.41 *b*	40.95 *bc*	69.75 *b*
	K326	2.61 *a*	127.86 *a*	3.33 *a*	38.48 *c*	96.54 *a*
	Altria1	2.26 *c*	113.01 *b*	2.57 *b*	44.56 *a*	74.40 *b*
	Altria2	2.28 *bc*	113.58 *b*	2.61 *b*	44.02 *ab*	75.68 *b*
43 lbs N	NC95	2.75 *a*	74.41 *b*	2.05 *a*	36.43 *b*	88.28 *a*
	LAFC53	2.37 *b*	65.51 *c*	1.56 *c*	42.45 *a*	67.00 *c*
	K326	2.65 *a*	82.26 *a*	2.19 *a*	38.29 *b*	94.28 *a*
	Altria1	2.28 *b*	78.53 *ab*	1.80 *b*	44.23 *a*	77.47 *b*
	Altria2	2.35 *b*	76.37 *b*	1.80 *b*	42.77 *a*	77.39 *b*
58 lbs N	NC95	2.62 *a*	53.90 *bc*	1.43 *b*	38.50 *b*	82.67 *b*
	LAFC53	2.20 *b*	50.00 *d*	1.11 *c*	45.82 *a*	64.39 *c*
	K326	2.66 *a*	63.68 *a*	1.70 *a*	37.78 *b*	98.44 *a*
	Altria1	2.44 *ab*	57.42 *b*	1.41 *b*	41.48 *ab*	81.71 *b*
	Altria2	2.46 *ab*	51.55 *cd*	1.28 *bc*	41.27 *ab*	74.15 *bc*
72 lbs N	NC95	2.64 *ab*	50.97 *a*	1.36 *ab*	38.24 *ab*	97.66 *ab*
	LAFC53	2.55 *abc*	39.63 *b*	1.02 *b*	39.61 *ab*	73.29 *b*
	K326	2.73 *a*	52.04 *a*	1.43 *a*	36.79 *b*	102.80 *a*
	Altria1	2.34 *c*	45.22 *ab*	1.06 *b*	42.91 *a*	76.08 *b*
	Altria2	2.38 *bc*	48.10 *ab*	1.15 *ab*	42.21 *a*	82.65 *ab*

Means followed by the same letter within a column grouped by N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 4.3. Analysis of variance for yield and grade index of two conventional and three low-nicotine varieties grown at four nitrogen rates combined over 2019 and 2020 (Southern Piedmont AREC).

Effect	Yield		Grade Index	
	F value	*P* value	*F* value	*P* value
Year	348.4798	<0.0001	8.5757	0.0066
Variety	26.1443	<0.0001	66.6065	<0.0001
N rate	36.0975	<0.0001	0.0579	0.8116
Year X Variety	2.4205	0.0596	1.1440	0.3600
Year X N rate	18.1176	0.0002	2.4401	0.1291
Variety X N rate	0.2426	0.9402	0.6931	0.6328

Table 4.4. Yield, grade index, and cured leaf chemistry of two conventional and three low-nicotine varieties grown with four nitrogen rates in 2019 and 2020 (Southern Piedmont AREC).

N Rate (lbs N/ac)	Variety	Yield (lbs/ac)	Grade Index (0-100)	Nicotine (%)	Reducing Sugars (%)
			2019		
29 lbs N	NC95	2098 *b*	58 *b*	2.84 *a*	15.27 *a*
	LAFC53	2066 *b*	17 *c*	0.63 *b*	12.10 *c*
	K326	2588 *a*	77 *a*	2.72 *a*	14.85 *ab*
	Altria1	2328 *ab*	42 *b*	0.53 *b*	13.27 *bc*
	Altria2	2329 *ab*	52 *b*	0.52 *b*	12.31 *c*
43 lbs N	NC95	2264 *bc*	65 *ab*	3.27 *a*	13.95 *a*
	LAFC53	1897 *c*	29 *c*	0.64 *c*	10.39 *b*
	K326	2672 *ab*	74 *a*	2.74 *b*	14.24 *a*
	Altria1	2700 *a*	55 *b*	0.60 *c*	10.61 *b*
	Altria2	2657 *ab*	73 *ab*	0.58 *c*	11.67 *b*
58 lbs N	NC95	2450 *bc*	62 *ab*	3.47 *a*	12.28 *a*
	LAFC53	2225 *c*	21 *c*	0.67 *c*	8.61 *b*
	K326	3016 *a*	74 *a*	3.04 *b*	11.84 *a*
	Altria1	2668 *ab*	49 *b*	0.54 *c*	7.94 *b*
	Altria2	2842 *a*	65 *a*	0.54 *c*	9.37 *b*
72 lbs N	NC95	2537 *c*	57 *ab*	3.80 *a*	10.69 *a*
	LAFC53	2658 *c*	32 *b*	0.77 *b*	8.01 *a*
	K326	3234 *a*	76 *a*	3.50 *a*	10.12 *a*
	Altria1	3028 *ab*	49 *ab*	0.61 *b*	7.29 *a*
	Altria2	2863 *bc*	65 *a*	0.61 *b*	7.57 *a*
			2020		
29 lbs N	NC95	3315 *b*	66 *a*	3.37 *a*	10.55 *a*
	LAFC53	2840 *c*	33 *b*	0.70 *b*	5.48 *bc*
	K326	3708 *a*	75 *a*	3.22 *a*	7.93 *ab*
	Altria1	3277 *b*	68 *a*	0.34 *b*	5.87 *bc*
	Altria2	3294 *b*	71 *a*	0.25 *b*	4.18 *c*
43 lbs N	NC95	3199 *b*	65 *ab*	3.65 *a*	8.40 *a*
	LAFC53	2817 *c*	19 *c*	0.28 *b*	4.11 *b*
	K326	3537 *a*	81 *a*	3.44 *a*	8.01 *a*
	Altria1	3377 *ab*	47 *b*	0.22 *b*	4.18 *b*
	Altria2	3284 *b*	62 *ab*	0.22 *b*	5.05 *b*
58 lbs N	NC95	3126 *bc*	64 *b*	3.40 *a*	9.12 *a*
	LAFC53	2900 *d*	33 *c*	0.38 *b*	6.55 *bc*
	K326	3693 *a*	82 *a*	3.32 *a*	7.82 *ab*
	Altria1	3330 *b*	69 *ab*	0.27 *b*	5.36 *c*
	Altria2	2990 *cd*	69 *ab*	0.27 *b*	6.45 *bc*
72 lbs N	NC95	3670 *a*	50 *a*	2.86 *ab*	7.36 *ab*
	LAFC53	2853 *b*	23 *b*	1.27 *bc*	4.79 *bc*
	K326	3747 *a*	72 *a*	3.33 *a*	8.20 *a*
	Altria1	3256 *ab*	60 *a*	0.21 *c*	3.00 *c*
	Altria2	3463 *ab*	69 *a*	0.29 *c*	3.71 *c*

Means followed by the same letter within a column grouped by N rate are not significantly different based on Fisher's protected LSD (α = 0.05).

Table 4.5. Analysis of variance for nicotine and reducing sugars of two conventional and three low-nicotine varieties grown at four nitrogen rates combined over 2019 and 2020 (Southern Piedmont AREC).

Effect	Nicotine		Reducing Sugars	
	F value	*P* value	*F* value	*P* value
Year	0.8589	0.3617	203.0406	<0.0001
Variety	252.4934	<0.0001	21.8266	<0.0001
N rate	5.0093	0.0331	43.5643	<0.0001
Year X Variety	2.2936	0.0714	1.4791	0.2270
Year X N rate	5.0269	0.0328	25.0880	<0.0001
Variety X N rate	0.7896	0.5658	0.5873	0.7095

Table 4.6. Information of parent lines, alkaloid pathways, and traits of two conventional and three low-nicotine varieties.

Variety	**Parent Line**	**Alkaloid Pathway**	**Trait**
NC 95	-	-	"Green"
K 326	-	-	"Quality"
LA FC53	NC 95	*Nic1&2*	"Greener longer"
Altria1	K 326	*Nic1&2*	-
Altria2	K 326	*Nic1&2*	"Bred for improved quality traits"

Table 4.7. Rainfall in inches at the Southern Piedmont AREC during the flue-cured tobacco growing seasons from 2019 and 2020 (Southern Piedmont AREC).

Southern Piedmont AREC Rainfall during the Growing Season (inches)

Day	May	June	July	Aug.	Sept.	Oct.	May	June	July	Aug.	Sept.	Oct.
	2019						**2020**					
1	0.00	0.00	0.03	0.01	0.00	0.01	0.03	0.00	0.00	0.15	0.01	0.00
2	0.00	0.01	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.00
3	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00
4	0.40	0.00	0.91	0.72	0.00	0.00	0.01	0.00	0.00	2.20	0.00	0.00
5	0.19	0.41	0.00	0.24	0.00	0.00	0.09	0.15	0.00	0.24	0.00	0.00
6	0.00	0.00	0.00	0.05	0.18	0.00	0.22	0.93	0.00	0.93	0.00	0.00
7	0.00	0.81	0.00	0.03	0.00	0.00	0.00	0.02	**1.00**	0.01	0.00	0.00
8	0.43	0.57	0.00	0.00	0.00	0.04	0.06	0.00	0.00	0.00	0.00	0.00
9	0.00	0.37	0.00	0.00	0.11	0.01	0.00	0.00	0.01	0.05	0.07	0.00
10	0.00	0.75	0.00	0.06	0.00	0.00	0.00	0.02	0.32	0.00	0.01	0.51
11	0.23	0.38	0.09	0.06	0.00	0.00	0.00	0.40	0.11	0.00	0.00	1.14
12	0.44	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.10
13	0.19	0.04	0.00	0.25	0.15	0.15	0.19	0.00	0.33	0.12	0.00	0.01
14	0.13	0.02	0.00	0.37	0.00	0.08	0.00	0.00	0.01	0.12	0.00	0.00
15	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.75	0.00	0.48	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	1.26	0.00	0.56	0.00	0.22	0.00	0.96
17	0.00	0.00	0.12	0.00	0.42	0.06	0.00	2.10	0.27	0.00	3.75	0.00
18	0.00	0.29	0.04	0.00	0.00	0.00	0.15	0.04	0.00	0.00	0.14	0.00
19	0.00	0.16	0.00	0.01	0.00	0.00	0.38	0.07	0.00	0.00	0.00	0.00
20	0.00	0.28	0.00	0.00	0.00	2.09	0.05	0.09	**1.00**	0.00	0.00	0.01
21	0.00	0.00	0.00	0.00	0.00	0.85	0.48	0.00	0.00	0.00	0.00	0.00
22	0.00	0.00	0.18	0.32	0.00	0.33	0.49	0.00	0.00	0.97	0.00	0.01
23	0.00	0.00	1.15	0.13	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.72	0.00	0.00	0.27
25	0.12	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.51	0.41
26	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
27	0.01	0.00	0.00	0.00	0.00	0.10	0.09	0.00	0.00	0.00	0.00	0.01
28	0.00	0.03	0.00	0.00	0.00	0.00	0.54	0.00	0.03	0.00	0.00	0.00
29	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.14	0.00	1.08	0.65
30	0.60	0.38	0.00	0.00	0.04	0.08	0.00	0.00	0.00	0.00	0.05	0.40
31	0.06	-	0.14	0.00	-	0.39	0.00	-	0.75	0.12	-	0.00
Rainfall Total	3.55	4.56	2.66	3.23	0.90	5.59	2.79	5.14	2.70	6.94	6.16	4.55

Irrigation events are indicated in blue and are not included in the monthly rainfall total.

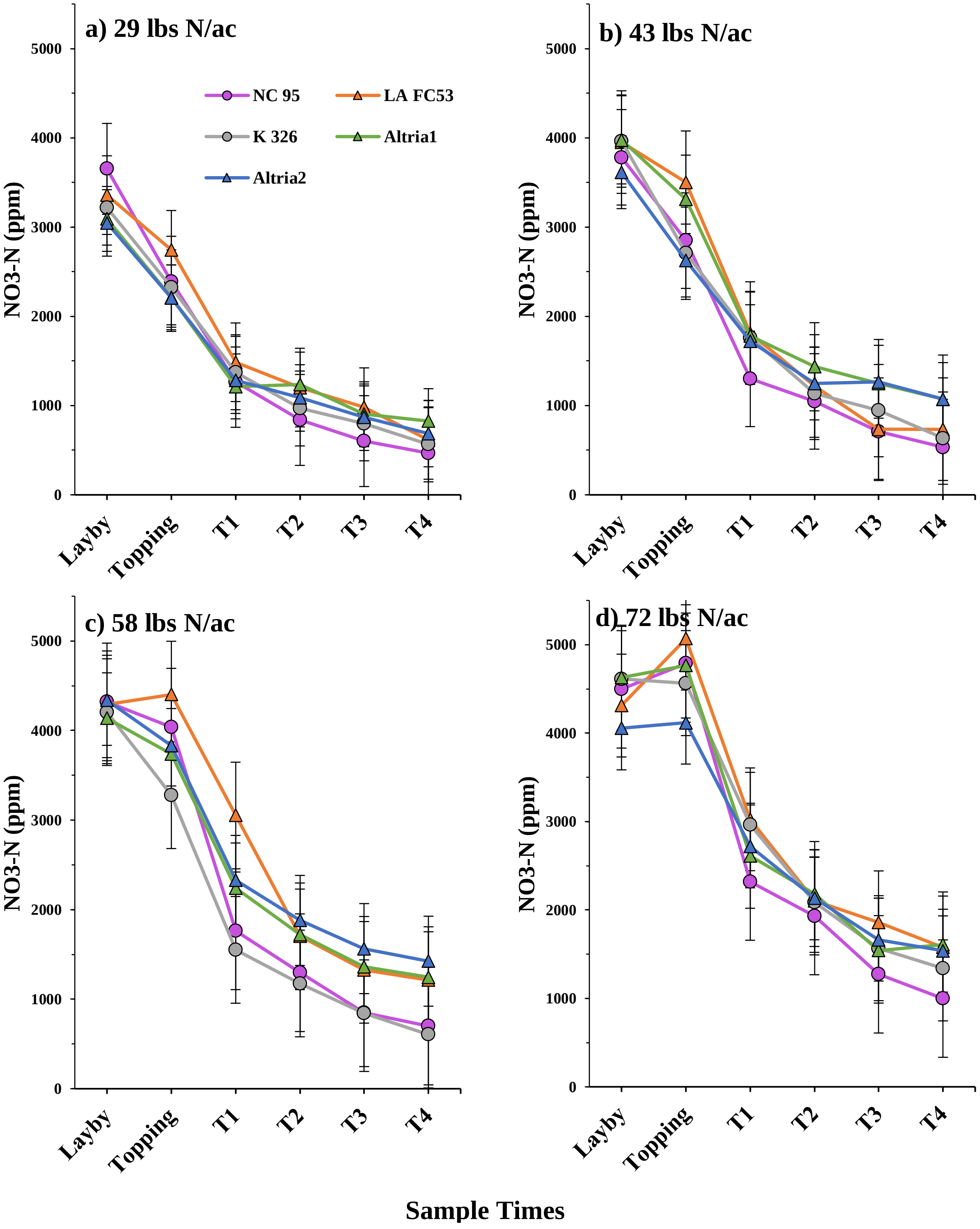


Figure 4.1 a-d. Petiole sap nitrate levels (a-d) of two conventional and three low-nicotine varieties grown with four nitrogen rates and sampled six times from the top of the plant. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

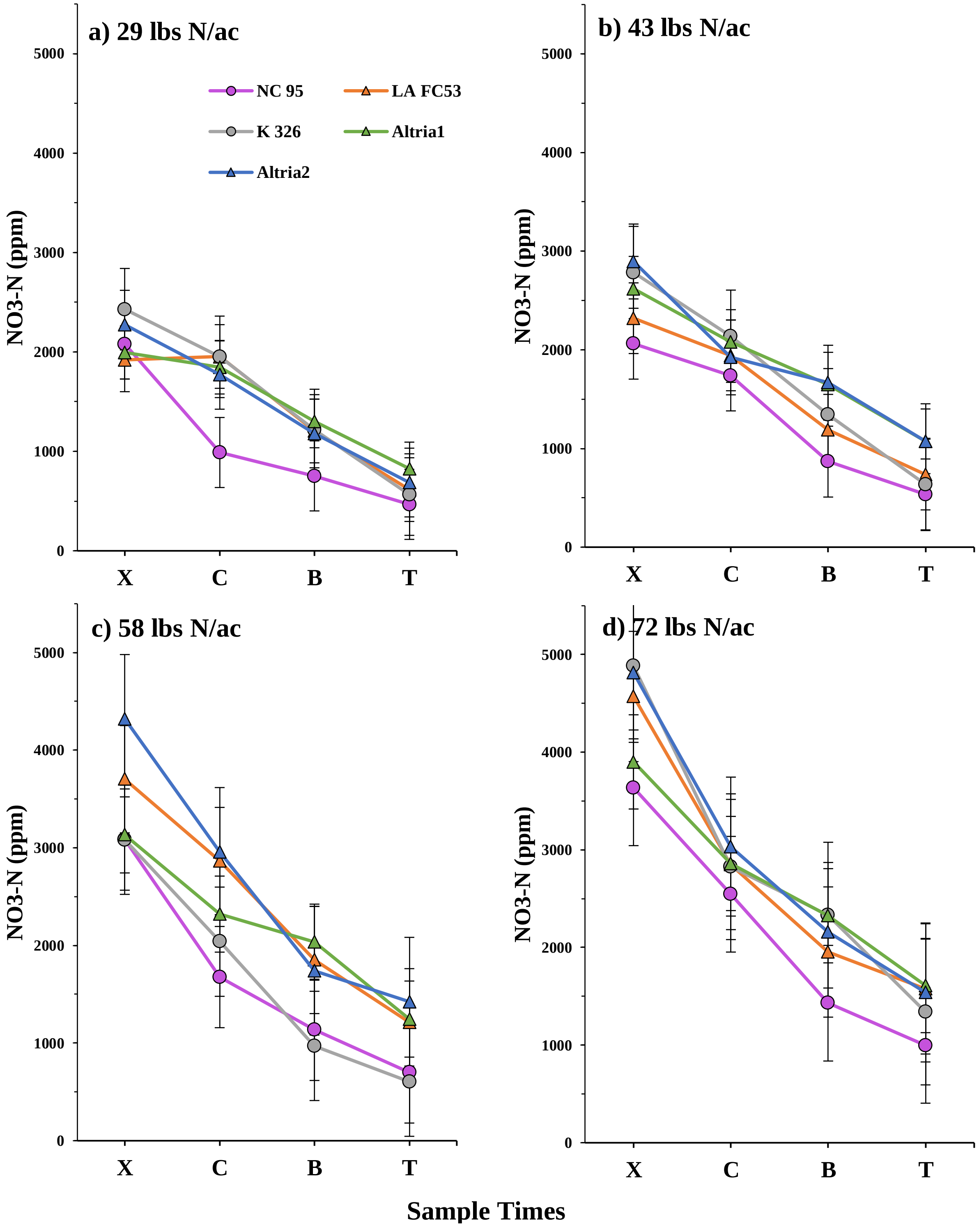


Figure 4.2 a-d. Petiole sap nitrate levels (a-d) of two conventional and three low-nicotine varieties grown with four nitrogen rates and sampled from the lugs (X), cutters (C), leaf (B), and tips (T) as harvested. Data are combined over 2019 and 2020 (Southern Piedmont AREC).

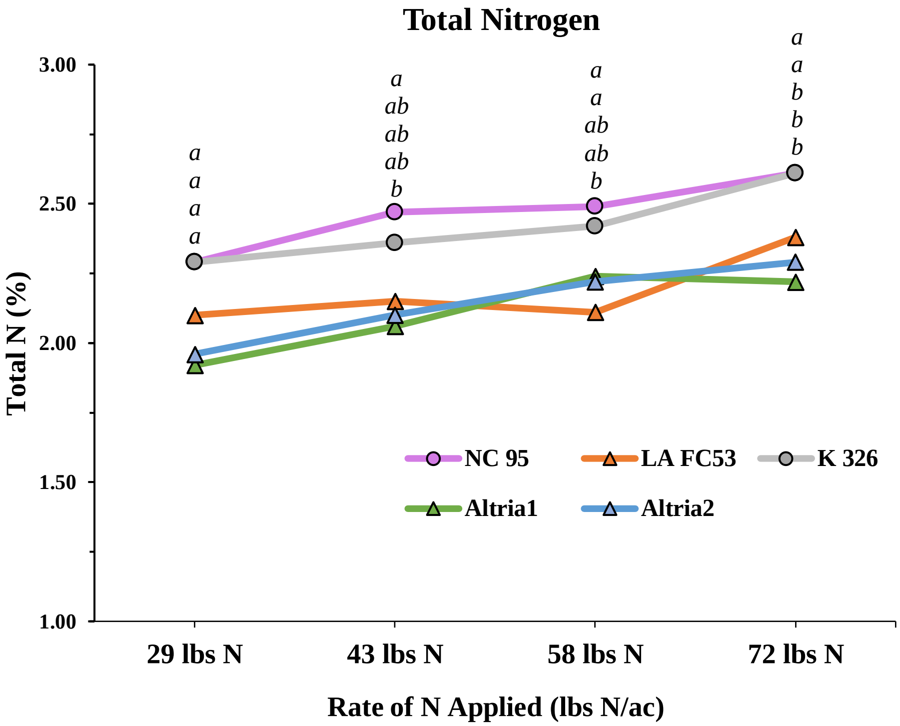


Figure 4.3. Composite whole-plant total nitrogen of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

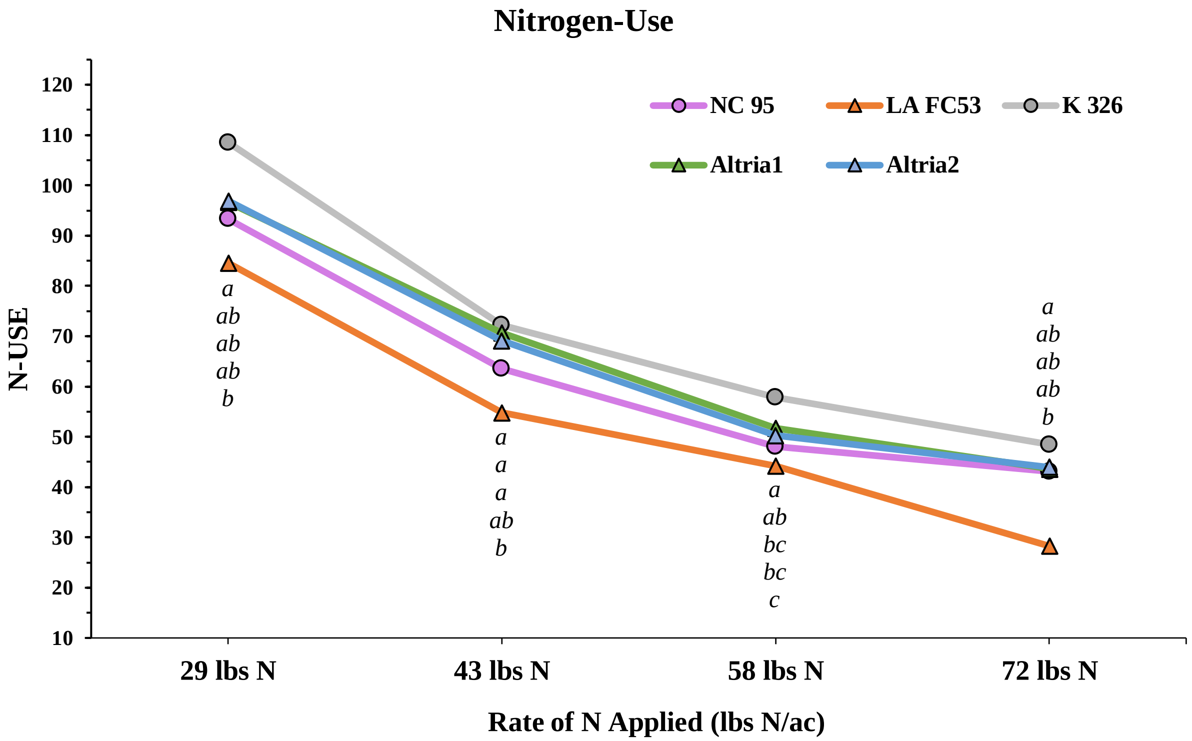


Figure 4.4. Nitrogen-use of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

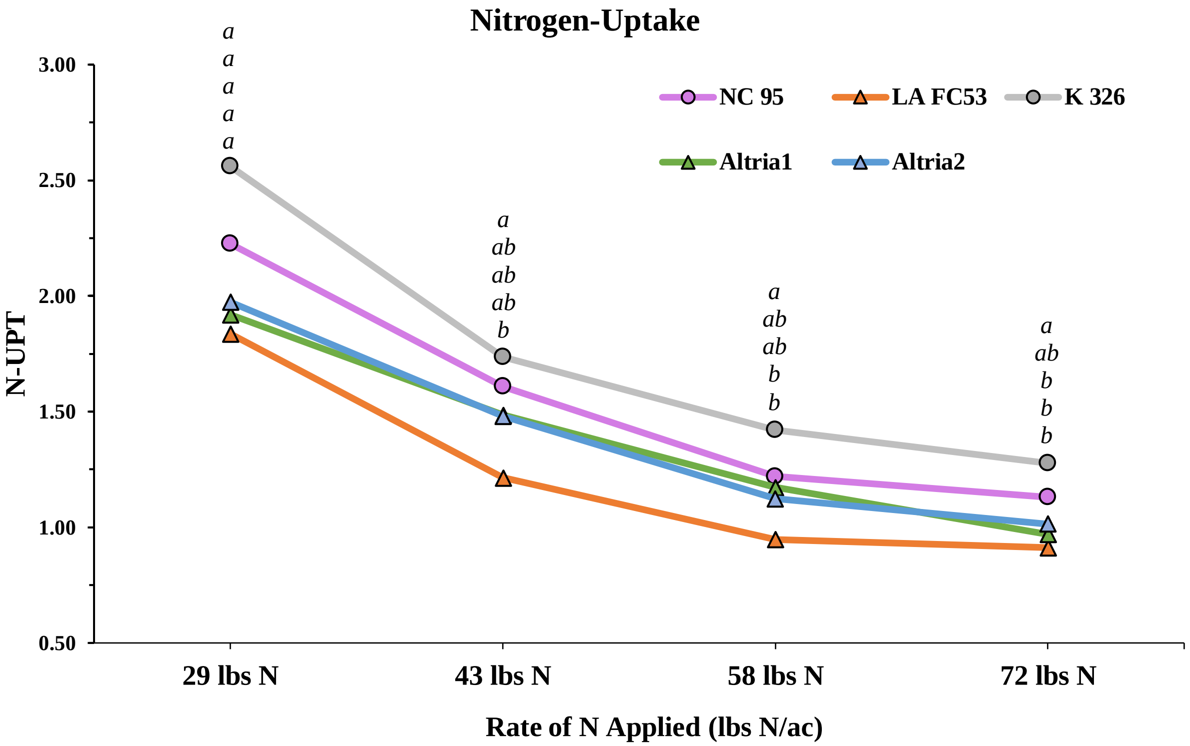


Figure 4.5. Nitrogen-uptake of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

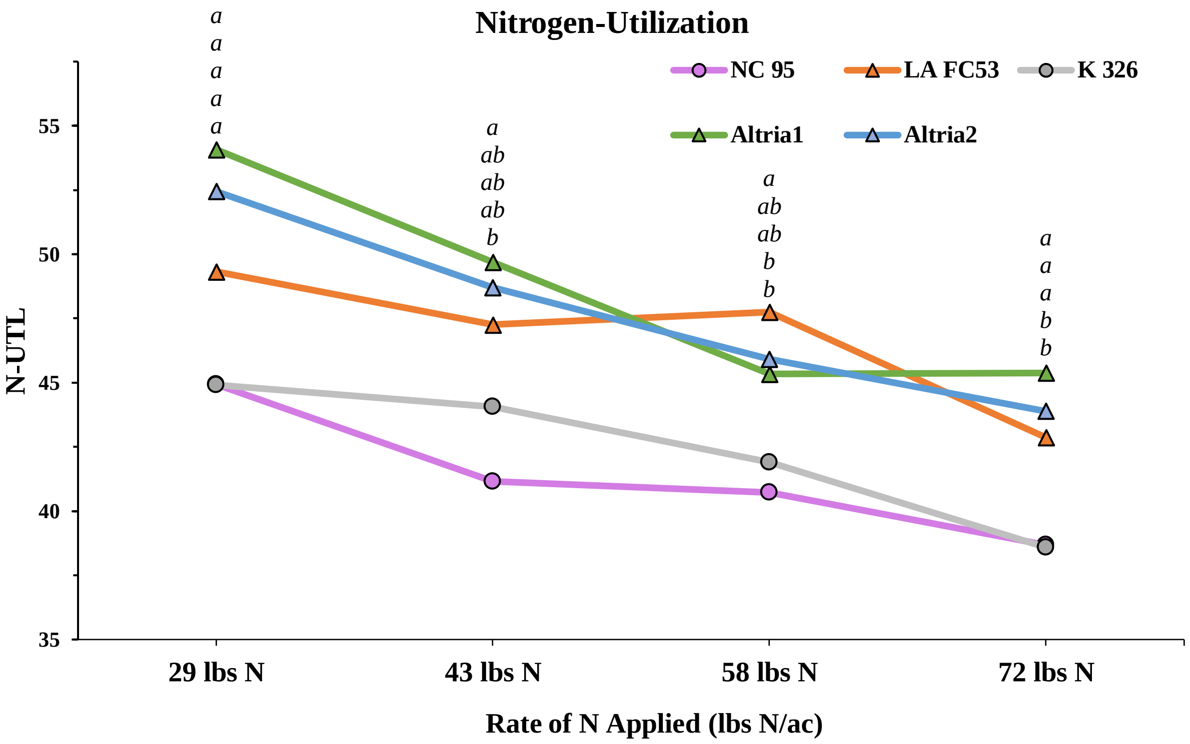


Figure 4.6. Nitrogen-utilization of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

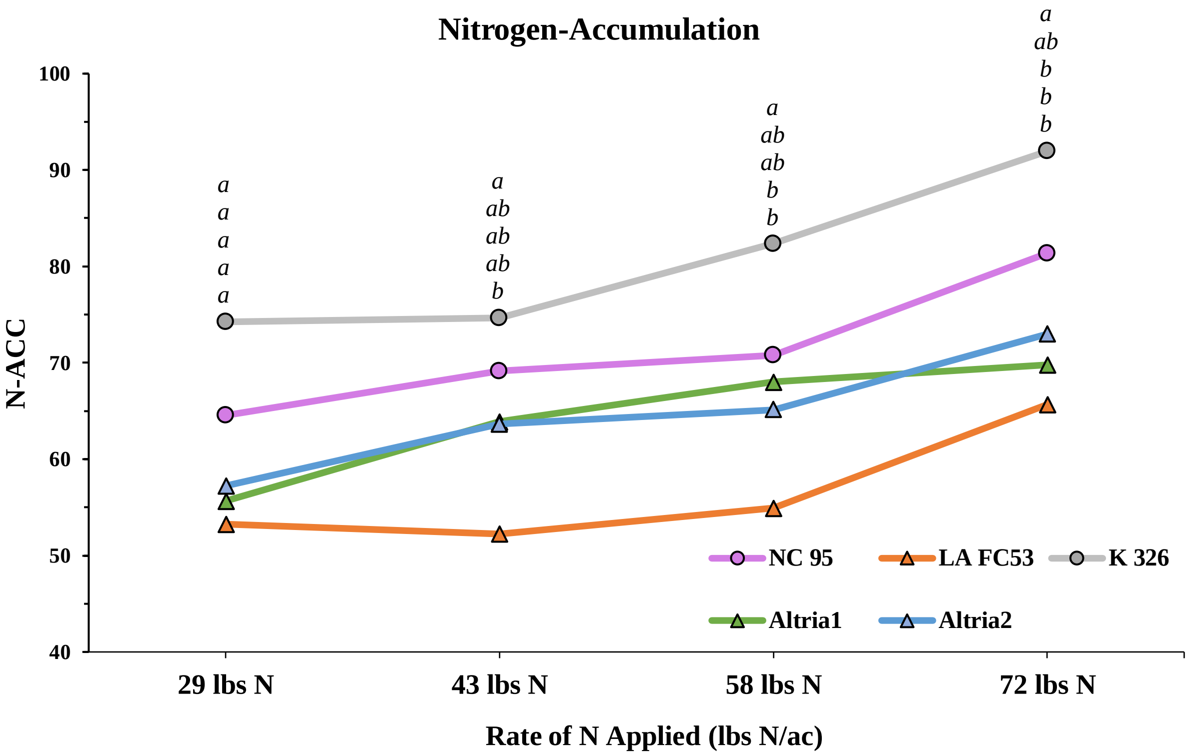
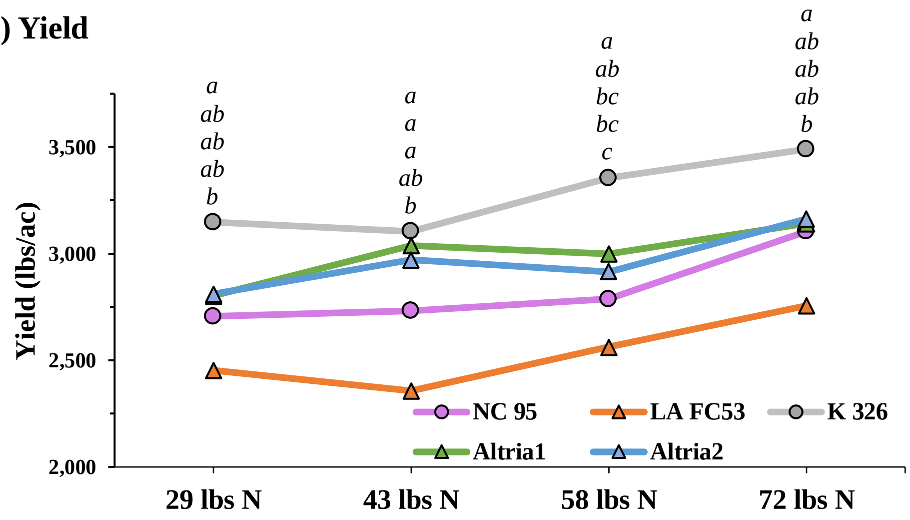


Figure 4.7. Nitrogen-accumulation of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

a) Yield



b) Grade Index

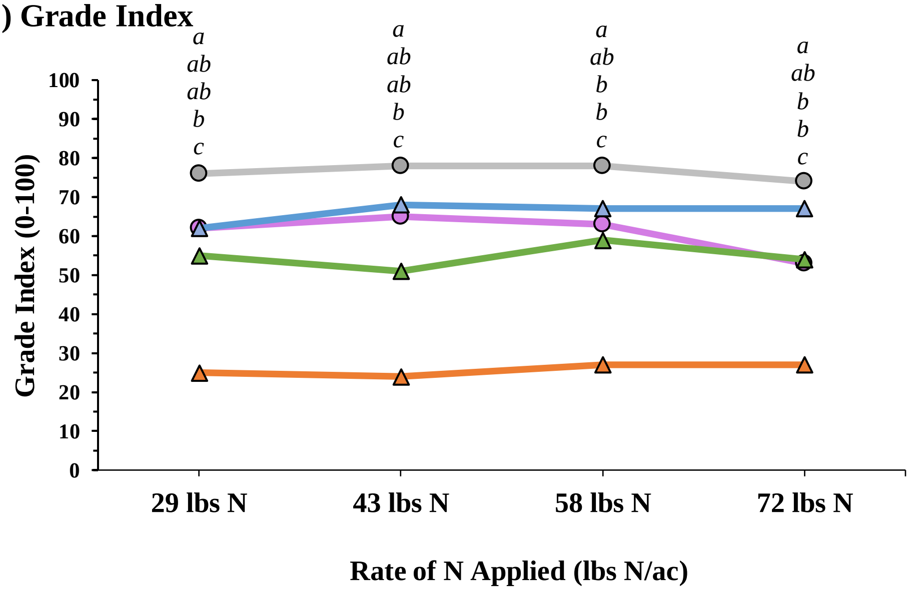
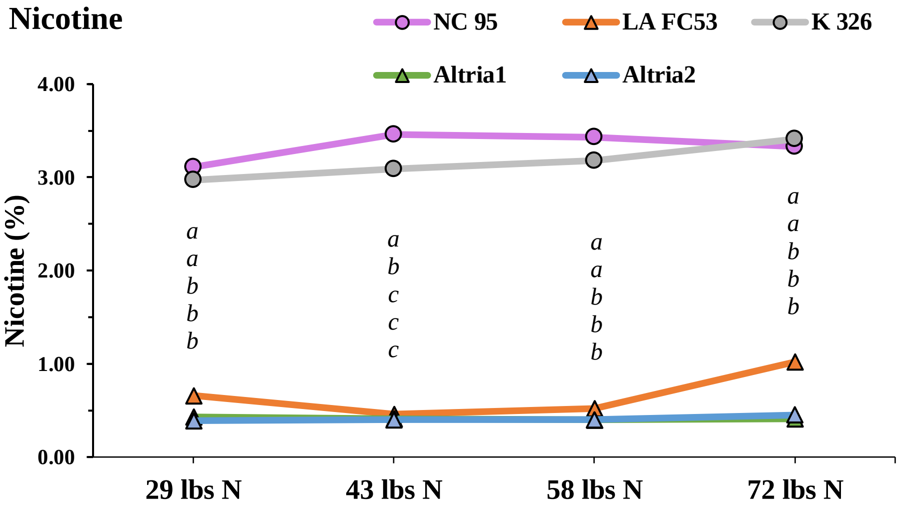


Figure 4.8 a-b. Yield and grade index of two conventional and three low-nicotine varieties grown at four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

a) Nicotine



b) Reducing Sugars

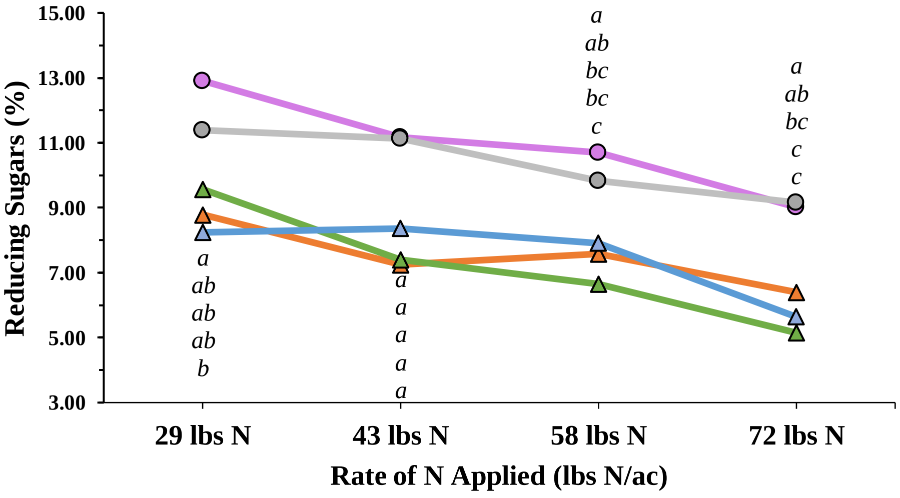
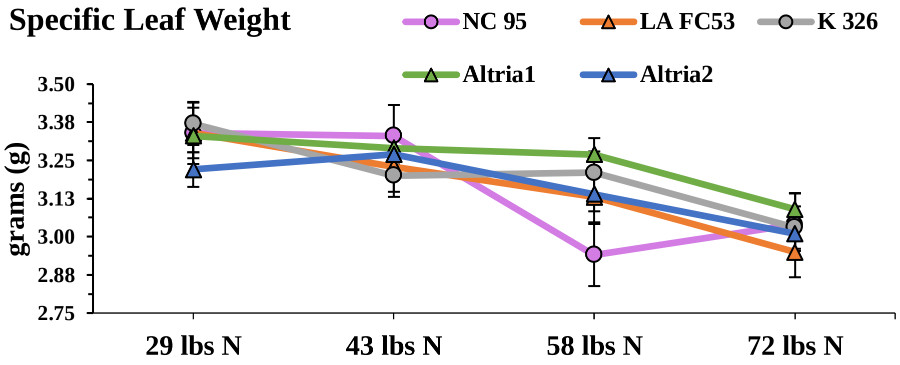
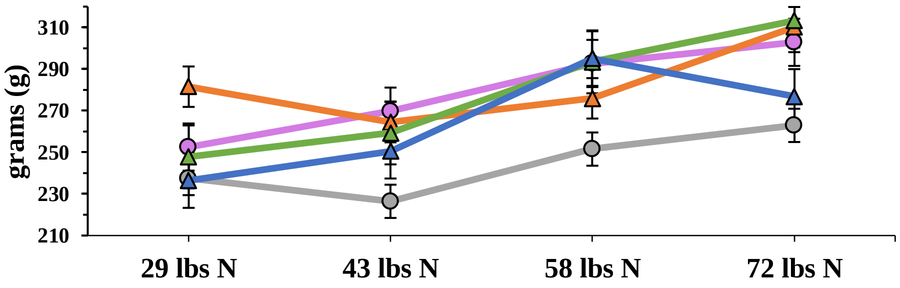


Figure 4.9 a-b. Composite whole-plant nicotine (a) and reducing sugars (b) of two conventional and three low-nicotine varieties grown at four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC). Mean separation letters correspond to the respective varieties (top to bottom) within a nitrogen rate grouping. Similar letters indicate means are not significantly different (LSD α = 0.05).

a) Specific Leaf Weight



b) Stalk Weight



c) Root Weight

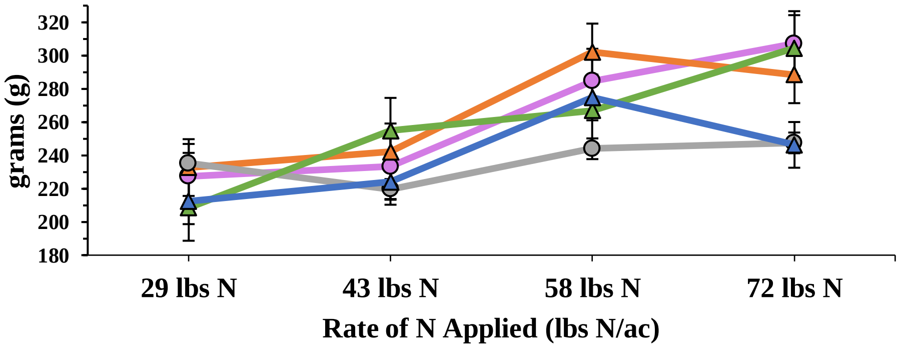


Figure 4.10 a-c. Specific leaf (a), stalk (b), and root weights (c) of two conventional and three low-nicotine varieties grown with four nitrogen rates averaged over 2019 and 2020 (Southern Piedmont AREC).

Chapter V

Conclusions and Future Direction

Conclusions

Increasing plant population in order to minimize points of nicotine synthesis at the root tips did not provide significantly lower levels of nicotine in either variety. These changes in planting population significantly increased yields in both varieties as the population was increased but grade index was not affected. This increase in yield by both varieties is most likely due to an increase in leaf count per acre.

Elimination of the main driver in nicotine dispersal throughout the plant, topping, was the only time in which both varieties significantly decreased nicotine levels. This treatment of an extended topping time and not topping plants at all had significant negative impacts on the yield of both varieties. Grade index was significantly impacted by an extended topping time during 2019, with the lowest values typically found in both varieties that were not topped. This treatment was not significant to grade index during 2020.

Changing the height at which plants are topped, based on total leaf count per plant, was studied as another method to dilute nicotine. Raising the height of topping would, in theory, dilute nicotine levels while a lowered height would increase the concentration but this study showed no significant effects from any treatment level. As expected, yield was significantly increased with a higher topping height in both varieties. This increased yield response is most likely due to an increase in leaf count per acre.

Overall, variety had the most consistent effect on nicotine levels in each study. Though levels of nicotine were lowered in plants not topped, the greatest impact was using LA FC53 in comparison to K 326. This effect from a change in variety was expected, as this variety was bred to have much lower levels of nicotine from the closing of specific pathways for synthesis. As anticipated, the greatest treatment level effect was from not topping plants at all. This process of topping is the main cause of nicotine dispersal throughout the plant; however, data collected also proves a serious negative impact on yield and cured leaf quality in both varieties.

In all parameters of nitrogen-use efficiency, both variety and N rate had significant effects on the levels therein. Important areas such as yield, grade index, and N-USE were consistently higher in K 326 but not always significantly. Newer, proprietary varieties ultimately showed no more than a few hundred pounds difference in yield per acre when compared to the top variety tested, K 326. The levels of grade index found in Altria2 only averaged a difference of approximately 10 when compared to that of K 326. Most likely due to its improved quality trait, Altria2 was always higher than NC 95, LA FC53, and Altria1. Though typically not significant, the N-USE of both Altria1 and Altria2 was only ever lower than K 326 and did not differ much from each other. Of the low-nicotine varieties tested, LA FC53 (the only publicly available low-nicotine variety) was the lowest in all of these major aspects. Conversely, recently developed low-nicotine varieties, Altria1 and Altria2, have shown significant potential to comply with FDA proposed levels of nicotine while also maintaining yield, grade index, and N-USE levels competitive to NC 95 and not much lower than K 326.

Future Direction

No work was done to evaluate smoking characteristics of cured leaf from these studies; however, more research should be conducted to quantify the smoking quality of these low-nicotine varieties. These important chemical and smoke measurements should be evaluated on low-nicotine cigarettes: menthol in cigarettes and smoke, total particulate matter, nicotine, CO, and cigarette puff count (McKenzie & Crawley, 1999). Similar to other packaged goods, cigarette consumers demand a consistent, high quality product to smoke (Fisher, 1999).

Research to discover alternate pathways for nicotine synthesis should be continued along with smoking characteristics. From this research, it could be suggested that with continued work on locating and understanding pathways for nicotine synthesis, more improved low-nicotine varieties could be developed. Even though some of these low-nicotine varieties provide promising agronomic results, more extensive work should be conducted to better develop these low-nicotine varieties on a commercial-scale.

References

Fisher, P. (1999). Tobacco Blending, In D. L. Davis & M. T. Nielsen (Eds.), *Tobacco Production, Chemistry and Technology* (pp. 346-352). Blackwell Science

McKenzie, J. L., & C. Crawley. (1999). Cigarette Production and Quality Assurance In D. L. Davis & M. T. Nielsen (Eds.), *Tobacco Production, Chemistry and Technology* (pp. 388-397). Blackwell Science