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Soil and Plant Monitoring of Nitrogen as Management Tools for Nitrogen
Fertilization and TSNA Reduction in Burley Tobacco

A Thesis
Presented for the
Master of Science
Degree
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William Hunter Frame
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Dedication

This thesis is dedicated to my former high school agriculture teacher, Mr. Tom Walton, whose leadership and passion for agriculture inspired me to pursue a career in production agriculture. He introduced to me soils and pushed me to be the best I could possibly be and for that I am grateful.

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Abstract

Burley tobacco (*Nicotiana tabacum* L.) has historically been fertilized with high nitrogen rates. This study was conducted to evaluate the effect of nitrogen rate on yield and total tobacco specific nitrosamine (TSNA) concentration. The experiment also evaluated the use of in season nitrogen monitoring techniques and how well they were able to predict yield and total TSNA concentration. In 2007, five preplant nitrogen treatments along with two sidedress nitrogen treatments were used. These included: 0, 67, 135, 202, 270 kg/ha nitrogen preplant and 0 and 67 kg/ha nitrogen sidedress. In 2008, there were only four preplant nitrogen rates with the two sidedress nitrogen rates. The 270 kg N/ha was removed for 2008. Also in 2008, four varieties were used and they were KT 204LC, TN 90LC, TN 90HC, and NC 7. Sidedress treatments were applied at three weeks after transplanting. The in season nitrogen monitoring techniques were the pre-sidedress nitrate test (PSNT), the Cardy® nitrate meter, and the CM 1000® chlorophyll meter. PSNT samples were taken before sidedress treatments were applied. Chlorophyll and Cardy® meter samples were taken at sidedress, topping, and harvest in 2007. A five weeks after transplant sampling date was added in 2008. Yields were optimized at 135 kg/ha total nitrogen rates. A quadratic plateau model was used to determine critical levels for PSNT and total nitrogen predicting yield and total TSNA. Relative yield plateaued at 0.953 with 204.8 kg/ha total nitrogen applied. A critical PSNT level of 46.4 ppm soil nitrate was established for burley tobacco. TSNA levels increased up to 135 kg N/ha and reached a plateau at 257.8 kg/ha total nitrogen. Total nitrogen applied was the best yield predictor out of the methods used to evaluate nitrogen status of burley tobacco. However analysis of PSNT for different soil types might prove to be a more reliable nitrogen management tool. Cardy® nitrate measurements did differ over the nitrogen rates, but there was no relationship between yield or TSNA and petiole nitrate

levels during the growing season. Chlorophyll measurements did not correlate to nitrogen rates or yield.

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Chapter 1

Introduction and General Information

Burley tobacco (*Nicotiana tabacum* L.) historically received high levels of nitrogen fertilization in order to achieve maximum yield. As a result of fluctuating prices of carbon based fossil fuels such as natural gas, nitrogen fertilizer prices have been unstable over the past two growing seasons. This concern, coupled with the lower prices in the post buy-out era, prompted researchers to reevaluate current nitrogen fertilizer recommendations in the traditional burley growing states. Previous research has indicated that optimum yields for burley tobacco can be reached at lower nitrogen rates than currently utilized by commercial producers.

Techniques for evaluating nitrogen status have been used successfully in other crops. One of these techniques is the monitoring of petiole nitrate in crops to more efficiently apply nitrogen fertilizer. A hand held nitrate meter, Cardy® nitrate meter by Minolta, has been successfully used to make nitrogen recommendations in specialty crops. Another technique used more in traditional row crops measures chlorophyll content of the leaf to monitor nitrogen status of crops. In the past, the standard for chlorophyll analysis was extraction which is done in a lab and plant tissue had to be destroyed. New non-destructive meters, like the CM 1000 chlorophyll meter®, are now available that give instantaneous results and multiple samples can run quickly. There are two types of chlorophyll measurements used with non-destructive meters: reflectance and absorbance. Another technique used is the pre-sidedress soil nitrate test (PSNT) to measure available nitrogen in the top fifteen centimeters of the soil profile. The PSNT has been very successful in differentiating corn production sites that will respond to sidedress nitrogen application from sites that will not respond. The use of these types of nitrogen management

tools in other crops gives hope that these techniques may prove to be useful in burley tobacco as nitrogen management tools.

Nitrogen fertilization of burley tobacco has also been linked to tobacco specific nitrosamines (TSNA) formation in cured leaf. TSNA are known carcinogens and tobacco companies are focused on eliminating TSNA in the cured leaf. There are many factors that contribute to TSNA formation, however recent advances in the screening process of seed stocks for low-conversion (LC) of nicotine to nornicotine in all new varieties has substantially reduced the TSNA content in recent varieties. Weather also plays a large role in formation of TSNA in the cured burley tobacco leaf. TSNA formation is a complex biological-biochemical reaction and a multitude of management techniques will have to be altered in order to eliminate TSNA in cured leaf and still maintain the quality desired by companies.

The primary objectives of this experiment are: 1) evaluate the current nitrogen fertilization recommendations for the major burley tobacco producing states, 2) evaluate the effectiveness of the Cardy® nitrate meter and CM 1000® chlorophyll meter for monitoring nitrogen status of burley tobacco at several dates within the growing season, 3) evaluate the ability of the Cardy® meter and CM 1000® to predict yields in burley tobacco, 4) determine, if possible, a critical level for PSNT and evaluate how PSNT is related to yield, 5) evaluate the effects of nitrogen fertilization on TSNA content, 6) determine if there is a relationship between Cardy® petiole nitrate levels and total TSNA content, and 7) determine the best predictor of yield and TSNA content of the techniques studied.

Chapter 2

Literature Review

Nitrogen Fertilization

Nitrogen is the soil absorbed element that plants need in the greatest amounts regardless of species, and is found in many plant cell components like amino acids and nucleic acids (Taiz and Zeigler, 2002). In many agricultural systems, nitrogen is the yield limiting nutrient in those systems. Burley tobacco requires large amounts of nitrogen to be applied in order to maximize yields. Previous research shows that burley tobacco yields increase with increasing nitrogen fertilization rates (MacKown and Sutton, 1998; MacKown et al., 2000; MacKown et al., 1999; MacKown and Sutton, 1997; Burns, 1986; Marchetti et al., 2006; Atkinson and Sims, 1973; Aycock and McKee, 1979; Jones and Tramel, 1979; Elliot and Court, 1978; Link and Terrill, 1982; Waynick, 2007). Given the high value of all types of tobacco compared to other agricultural crops, nitrogen has historically been viewed as a cheap input to ensure maximum yields which has led to producers applying higher than recommended rates. The recommended nitrogen rates for the major burley tobacco producing states are 168-308 kilograms nitrogen per hectare (kg N/ha) in Kentucky, 168-224 kg N/ha in Tennessee, 196-224 kg N/ha in Virginia, and 168-280 kg N/ha in North Carolina (Pearce et al., 2009; Peek et al., 2008; Hoyt., 2008). Kentucky nitrogen recommendations are based on field rotations and soil drainage class. North Carolina incorporates yield potential into the nitrogen recommendations. This is why Kentucky and North Carolina have larger ranges in the recommended nitrogen rates than Tennessee and Virginia. Using nitrogen rates that exceed the recommended rates can have adverse effects on the environment, be unprofitable for the producer, and produce cured leaf with undesirable

characteristics. Using large amounts of nitrogen fertilizer on tobacco can result in cured leaf that is high in nitrates, nitrites, and alkaloids (MacKown et al., 1999; MacKown and Sutton, 1997; Burns, 1986; Marchetti et al., 2006; Atkinson and Sims, 1973; Aycock et al., 1979, Jones and Tramel, 1979; Broaddus et al., 1965; Hamilton et al., 1982; Elliot and Court, 1978; McKee, 1978; Link and Terrill, 1982; Bush et al., 2001). MacKown et. al (2000 and 1999) has shown that increases in cured leaf yields decrease with increasing nitrogen rates. The leveling off or plateauing of yields in burley tobacco at high rates of nitrogen fertilization has also been described as a sigmoidal curve by Sims and Atkinson (1973). So, producers are not seeing an increase in yield to justify using more nitrogen fertilizer beyond a specific rate. Atkinson and Sims (1973) found that above 179 kg N/ha there was no significant increase in burley tobacco yields. This is affirmed by Link and Terrill (1982) who also found that 170 kg N/ha was adequate for burley tobacco production in southwest Virginia. In flue-cured, Maryland, and Virginia dark-fired tobacco, research has shown that a reduction in the quality of the cured leaf will occurred in situations where excess nitrogen was applied (Marchetti et al., 2006; Burns, 1986; Jones and Tramel, 1979; Elliot and Court, 1978). Link and Terrill (1982) suggested that excessive rates of nitrogen fertilization may reduce the value of cured leaf in burley tobacco, but their results showed no measured difference in \$/kg of cured leaf at different nitrogen rates. Historically, quality of burley tobacco cured leaf has not been influenced by nitrogen fertilization rates. Atkinson et al. (1977) and MacKown and Sutton (1997) has noted that nutrient uptake by burley tobacco is very slow at the beginning of the growing season and may take several weeks for the plant to starting utilizing preplant nitrogen fertilizer. MacKown and Sutton (1997), MacKown and Sutton (1998), MacKown et. al (1999), and MacKown et al. (2000) suggests that

split applications or alternative methods of managing nitrogen status of burley tobacco may reduce the environmental impact and increase nitrogen efficiency without compromising yield.

Pre-sidedress Nitrate Test (PSNT)

The nitrogen cycle has been well documented and studied in order to understand how nitrogen is recycled in natural and human modified environments. In soils nitrogen can either be plant available or sequestered in organic forms that are not available to plants. The microbial mediated process by which organic forms are converted to plant available forms of nitrogen is called mineralization (Brady and Weil, 2002). The reverse process of mineralization is immobilization. The main form of nitrogen formed from mineralization is ammonium (NH_4^+) which can undergo ammonia volatilization or nitrification. Ammonia volatilization results in the production of ammonia gas (NH_3), while nitrification produces nitrite (NO_2^-) and nitrate (NO_3^-) that are also plant available (Brady and Weil, 2002).

Nitrogen fertilizers are applied to agricultural crops in order to make up for the difference between what mineralization and other soil processes can supply to the plant and the amount of nitrogen the plant needs in order to maximize yields (Magdoff, 1991). Magdoff (1991) developed a soil nitrate test for corn in which soil samples from the 0 to 30 cm depth are taken when the corn plants are 20-30cm in height, in order to determine how much plant available nitrogen is currently in the soil profile. Because of the small size of the corn at the time of sampling, the test was dubbed the pre-sidedress nitrate test (PSNT) because after receiving the results you could still apply a sidedress application of nitrogen fertilizer. Magdoff originally intended the test to be used in the northeast United States on fields where manures were applied the previous year, or legumes have been grown in the past three years, in order to more

accurately account for nitrogen being released by mineralization when soil temperatures are warm enough for microbial processes to occur (Magdoff, 1991; Andraski et al., 2002; Meisinger et al., 1992). The PSNT was designed to prevent losses of nitrogen through leaching and entering ground or surface waters by optimizing fertilization rates to match the uptake potential of the crop.

Previous research has shown that soil nitrate concentrations increase with increasing preplant nitrogen fertilizer applications (Magdoff, 1991; MacKown et al., 1999; Evanylo and Alley, Magdoff et al., 1990; Meisinger et al., 1992; Heckman et al., 2002; Krusekept et al., 2002; Waynick, 2007; Ma et al., 2005; Klausner et al., 1993). Andraski et al.(2002) have shown that using the PSNT could potentially lower nitrogen rates by an average of 90-102 kg N/ha on fields where manure has been applied or legumes have been grown in the previous three years. Also, critical soil $\text{NO}_3\text{-N}$ levels have been found to be in the range of 20-30 parts per million (ppm) $\text{NO}_3\text{-N}$ in the soil for corn and very similar levels have been found for other crops such as fall cabbage, sweet corn, and tomatoes (Magdoff, 1991; Magdoff et al., 1990; Binford et al., 1992; Heckman et al., 2002; Krusekopt et al., 2002; Rozas et al., 2000). Conrad (2002) found that yields of Maryland tobacco still increased with each higher nitrogen rate when PSNT values were 13-20 ppm $\text{NO}_3\text{-N}$ following crimson clover, but saw no significant yield responses on plots previously in hairy vetch with PSNT values of 14-26 ppm $\text{NO}_3\text{-N}$. MacKown et al. (1999) suggested critical PSNT values of 48 ppm and 88 ppm $\text{NO}_3\text{-N}$ for two different types of soils in Kentucky where burley tobacco was grown. Waynick (2007) also showed that yield responses occurred when PSNT values were 40 ppm $\text{NO}_3\text{-N}$ up to 80 ppm $\text{NO}_3\text{-N}$, though at higher levels the responses were less frequent in the treatments. The PSNT samples taken in Waynick's study were to a depth of 15 cm, not the 30 cm recommended by Magdoff. This research shows that

PSNT critical values are likely to be higher for burley tobacco than other crops. Zartman's (1976) research has shown that accumulation of nitrogen by burley tobacco was proportional to the concentration of $\text{NO}_3\text{-N}$ in the soil solution.

Tobacco-Specific Nitrosamines (TSNA)

Burley tobacco is hung in barns or other modified structures after harvest in order to cure the tobacco. It is during the curing process that tobacco-specific nitrosamines (TSNA) are formed (Bush et al., 2001; Wahlberg et al., 1999; Burton et al., 1989; Wiernik et al., 1995; Djordjevic et al., 1989). TSNA are formed via a nitrosation reaction involving nitrite derived species and secondary and tertiary alkaloids present in the leaf at curing (Bush et al., 2001; Wahlberg et al., 1999; Rundolf et al., 2000; MacKown et al., 1984; Burton et al., 1994; Wiernik et al., 1995; Burton et al., 1992). Figure 2.1 shows the nitrosation reactions with the major alkaloids found in tobacco and the nitrosamines formed by each reaction. Nitrosamines have recently become an important topic in tobacco because they have been proven to be carcinogens. Nitrosamines are found in water, beverages, foodstuffs (e.g. fried bacon), and tobacco products, but the concentration of nitrosamines in tobacco products is usually higher than in other products (Wahlberg et al., 1999). The major TSNA are N-nitrosornicotine (NNN), N-nitrosoanabasine (NAB), nitrosoanatabine (NAT), and 4-methylnitrosoamino-1-(3-pyridyl)-1-butanone (NNK). Three less frequently occurring nitrosamines: 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), 4-(methylnitrosoamino)-4-(3-pyridyl)-1-butanol (iso-NNAL), and 4-(methylnitrosoamino)-4-(3-pyridyl)-butanoic acid (iso-NNAC) (Wahlberg et al., 1999; MacKown et al., 1984; Wiernik et al., 1995). NNN is the N-nitrosoamine of greatest concern in burley tobacco.

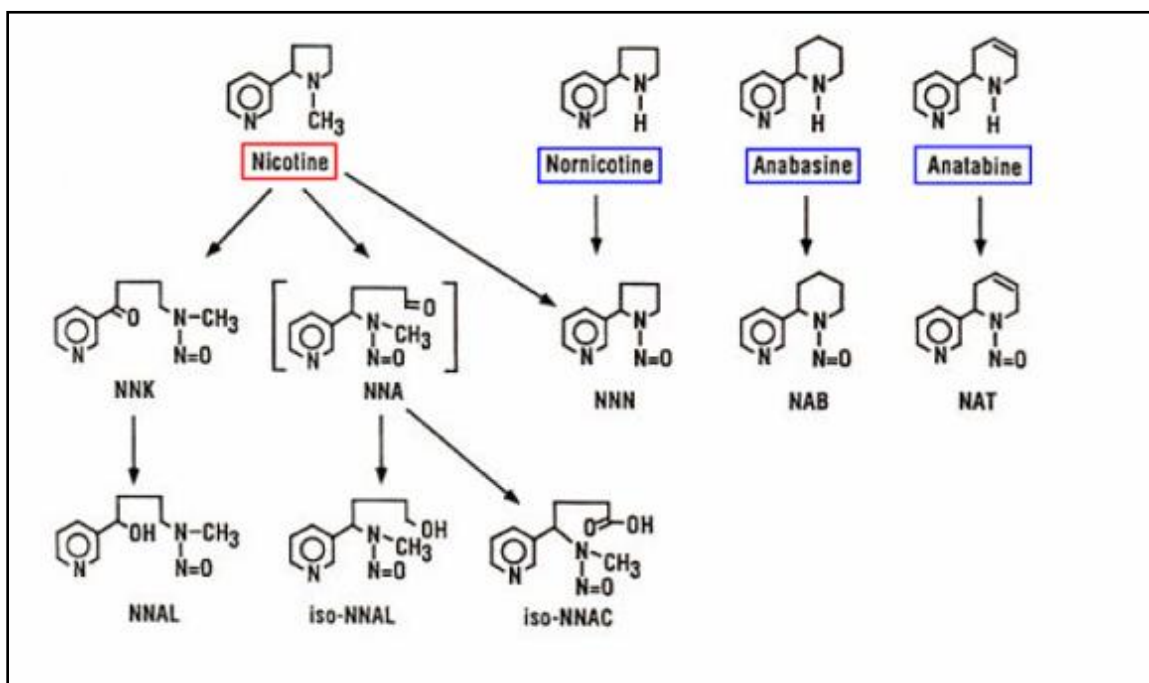


Figure 2.1 Tobacco-specific nitrosamine formation (Anonymous, 1999)

Knowing some of the basic research and science on how nitrosamines are formed chemically, we must look at what dictates where and to what extent TSNA will accumulate. Nitrite, along with nitrite derived species, and alkaloids are the reactants in the TSNA reaction. Nitrite formation is thought to be the product of bacteria reducing nitrate during the yellowing phase of the curing process (Bush et al., 2001; Wahlberg et al., 1999; Wiernik et al., 1995; Hamilton et al., 1982; Rundolf et al., 2000). The bacteria are thought to be intercellular and are able to reduce nitrate at the highest rates when cell wall integrity fails during the yellowing to browning phase of the curing process in burley tobacco. Previous studies have shown that the midrib of tobacco leaves have higher concentration of nitrate than lamina tissue (Broaddus et al., 1965; Bowman, 1972; MacKown et al., 1990; Wiernik et al. 1995). Nitrate levels within the tobacco plant generally decrease with increasing stalk positions, so the bottom of the plant has

higher nitrate levels than the top of the plant (Wiernik, 1995; Broaddus et al., 1965; Bowman, 1972). Nitrate reductase, which is found naturally occurring in plants, is the enzyme microbes use to reduce nitrate to nitrite. Burton et al. (1992, 1994) and MacKown et al. (1990) found that nitrite may be a better predictor of TSNA accumulation than alkaloid or nitrate concentrations, and that reduction of nitrate to nitrite is the rate limiting step in TSNA formation. It has been shown that alkaloids increase with higher nitrogen fertilization rates, and since the nitrite is nitrate-derived nitrogen fertilization practices do influence TSNA accumulation (MacKown et al., 1999; MacKown and Sutton, 1997; Burns, 1986; Marchetti et al., 2006; Atkinson and Sims, 1973; Aycock et al., 1979, Jones and Tramel, 1979; Broaddus et al., 1965; Hamilton et al., 1982; Elliot and Court, 1978; McKee, 1978; Link and Terrill, 1982; Bush et al., 2001; Wahlberg et al., 1999; Wiernik et al., 1995; Hamilton et al., 1982; Rundolf et al., 2000). Nitrite and nicotine are found in higher concentrations in leaf lamina than midrib, which is opposite of nitrate accumulation (Burton et al. 1992). Waynick (2007) showed that TSNA concentrations are higher in the midrib of tobacco leaves than leaf lamina, which is supported by previous work (Bush et al., 2001; Wahlberg et al., 1999; Wiernik et al., 1995). As with any biological reaction, temperature and moisture play a key role in TSNA accumulation in tobacco. Tobacco cured at higher temperatures and relative humidity can approach concentrations of 1mg/g of dry tissue, which is 400 times greater than the concentration of nitrosamines typically found in burley tobacco (Burton et al., 1989). Burton et al. (1989) showed that stored tobacco accumulated higher levels of TSNA at high temperatures and high humidity conditions. Bush et al.(2001) stated that different methods of curing (barns vs outdoor structures) can result in significant differences in TSNA concentrations, since modified structures typically have higher humidity and temperatures. Temperature and humidity conditions that decrease TSNA formation typically

result in tobacco that is not marketable for cigarette production. Rundolf et al. (2000) conducted a study looking at potential nitrite scavengers as inhibitors to TSNA formation and found that caffeic acid, dihydrocaffeic acid, ferulic acid and catechin were able to inhibit NNN formation in solution as well as a tobacco matrix. Other studies have shown that ascorbic acid has the ability to reduced TSNA formation in conditions similar to those found at curing (Rundolf et al., 2000; Bush et al., 2001; Wahlberg et al., 1999).

Chlorophyll Meter

The “gold standard” for chlorophyll determination has been extraction of pigments into a solvent followed by spectrophotometric analysis to determine the concentration of chlorophyll (Markwell, 2002). Recently two types of meters have been developed to estimate chlorophyll content in plants without having to destroy the plant and extract the pigments. The two types of meters are transmittance/absorbance (T/A) and reflectance (Murdock et al., 2004, Richardson et al., 2002). Chlorophyll has been focused on because it is highly correlated to nitrogen concentration within leaves of plants (Blackmer and Schepers, 1994; Ma et al., 2005; Peng et al., 1995; Wood et al., 1992a; Wood et al., 1992b; Piekielek et al., 1995). The majority of the work that has been done with chlorophyll meters has been with the T/A meters because they have been available longer than the reflectance meters. T/A meters measure how much light is transmitted through or absorbed by a small section of a single leaf and typically utilize their own light source. Reflectance meters measure incoming sunlight at different wavelengths and then have an optical sensor that measures those same wavelengths being reflected by the leaf surface or over the canopy surface. Most meters on the market utilize one or more wavelengths in the Red spectrum and one or more wavelengths in the Near-Infrared spectrum, using ratios of Red/Near-

Infrared to calculate with chlorophyll indices. There are many indices used to quantify reflectance measurements: two of these are the Normalized Difference Vegetative Index (NDVI) and Relative Vegetative Index (RVI) (Ma et al., 2001). Both types of meters allow chlorophyll content to be measured rapidly and multiple times within plots or even plants (Murdock et al., 2004; Blackmer, 1994; Ma et al., 1996; Ma et al., 2001; Blackmer and Schepers., 1995; Richardson et al., 2002; Markwell, 2002). Blackmer and Schepers (1994) was able to use a T/A meter and reflectance meter to determine whether corn was deficient of nitrogen. Ma et al. (2005) compared two types of chlorophyll meters to the PSNT and found that the chlorophyll meters were able to discriminate between preplant fertilizer treatments in all years. An adequately fertilized section of field (reference strip) should be measured and use to normalize the chlorophyll data from the rest of the field in order to make accurate nitrogen fertilization recommendations (Murdock et al., 1997; Varvel et al., 2007; Blackmer et al, 1995; Blackmer et al., 1994; Follet et al., 1992; Schepers et al., 1992; Minotti et al., 1994; Wood et al., 1992). Also reflectance meters, such as the Spectrum Technologies CM 1000® chlorophyll meter, are affected by the angle of the meter to the leaf surface and the time of day the reading is taken (Murdock et al., 2004; Ma et al., 1996). Murdock et al. (2004) suggests that readings with the CM 1000 be taken between 10am and 2pm to maximize light intensity and at a 90° angle to the leaf surface with the sun at your back to obtain the most consistent readings. Chlorophyll meter readings are affected by plant variety, cultural practices, and other climatic factors (Follet et al., 1992; Ma et al., 2001; Schepers et al., 1992; Wood et al., 1992). Reeves et al. (1993) used a chlorophyll meter on wheat and found that 81% of the variation in yield could be accounted for by the chlorophyll meter and dry matter accumulation. Blackmer and Schepers (1994) found that chlorophyll meter readings were highly correlated to corn yields at the R5 growth stage.

Chlorophyll meters may be a better indicator of nitrogen status in various crops because they reach a plateau in adequately fertilized areas suggesting that they are not affected by luxury consumption, unlike traditional total nitrogen measurements (Blackmer and Schepers, 1994; Blackmer and Schepers, 1995; Schepers et al., 1992). Chlorophyll meters have shown potential to be very useful in nitrogen fertilization management in many crops.

Cardy Nitrate Meter

The Cardy® nitrate meter is made by Horiba Co., Japan and can be used to directly measure soil or plant sap nitrate concentrations. The company also makes meters that detect sodium (Na^+) and potassium (K^+). The Cardy meter has been evaluated extensively on vegetables and other high value specialty crops as a way to manage nitrogen fertilization more efficiently (Westerveld et al., 2003; Westerveld et al., 2007). Constable et al. (1991) conducted research on cotton showing that as the rate nitrogen applied increased, the concentration of petiole nitrate increased at all sampling dates. However, Constable et al. used traditional methods of measuring nitrate in cotton petioles. Gardner and Roth (1989) were able to have an $R^2 = 0.75$ between petiole nitrate concentrations in broccoli and yield of broccoli over a three year study. They also used dried petioles and the nitrate sensitive electrode method for petiole nitrate determination. Kubota et al. (1996) and Westcott et al. (1993) compared the dried petiole nitrate to Cardy® nitrate measurements resulting in evidence that the Cardy® nitrate measurements were highly correlated to nitrate sensitive electrode measurements in broccoli and potato. Both Kubota et al.(1996) and Westcott et al.(1993) developed equations for broccoli and potatoes that allowed producers to convert the Cardy® meter measurements into dried petiole measurements

so that the numbers can be used with current guidelines for nitrogen recommendations in those crops. Kubota et al. (1996) developed equations that had a R^2 equal to 0.79 and Westcott et al. (1993) had a correlation coefficient for their equation of $r = 0.96$. Westerveld et al. (2003) determined that the Cardy® meter readings were a good indication of soil and sap nitrate concentrations in cabbage, carrots, and onions. Fontes and Ronchi (2002) used the Cardy® nitrate meter in research on tomatoes and concluded that sap nitrate has a strong quadratic relationship with nitrogen rate in soil, $R^2 = 0.79$. Research conducted on artichokes by Rodrigo et al. determined that the Cardy meter could be used as a nitrogen management tool; however, freezing petiole samples before analysis significantly reduced the Cardy meter readings. Westerveld et al. (2007) conducted research on carrots and found that there were differences in Cardy meter readings between two varieties of carrots. Westerveld et al. (2007) found that petiole nitrate increased with increasing nitrogen, indicating that some luxury consumption was occurring. Westerveld et al. (2007) concluded that the Cardy nitrate meter would not be a suitable nitrogen recommendation tool because of the variation seen across years and soil. Caldwell (2007) used the Cardy® nitrate meter on burley tobacco at three different sampling dates (four, six and eight weeks after transplant) over multiple irrigation, fertigation, and nitrogen treatments. At four weeks Caldwell found no differences in Cardy® meter reading across nitrogen rates. At six weeks Caldwell observed a drop in nitrate concentration with decreasing preplant nitrogen fertilizer rates. Also, the eight week after transplanting petiole samples were correlated to TSNA accumulation with a correlation coefficient of $r = 0.56$ (Caldwell, 2007).

Chapter 3

Materials and Methods

General Overview

Nine studies over two years and five locations in four states were conducted in order to evaluate nitrogen fertilization rates and timing of nitrogen fertilization in burley tobacco. In 2007, nitrogen fertilizer was applied at five preplant rates and two sidedress rates for a total of ten nitrogen rates. Studies in 2007 were conducted at Greeneville, TN; Springfield, TN; Glade Springs, VA; Lexington, KY; and Dixon Springs, IL. Studies were conducted in 2008 at all previous mentioned location except for Dixon Springs, IL. The studies in 2008 were expanded at four locations to evaluate the potential difference in response of four varieties. Tobacco specific nitrosamines (TSNA) data, grade index, pre-sidedress nitrate soil samples (PSNT), leaf chlorophyll data, petiole nitrate and yield data were taken for all studies.

Experimental site and soil description at Greeneville (GR)

In 2007 and 2008, Greeneville experimental sites were located at the University of Tennessee's Research and Education Center at Greeneville. The research station is located in Greene County, Tennessee, which is in the northeastern portion of Tennessee. Portions of Greene County can be found in the Great Valley and Appalachian upland physiographic regions of Tennessee (USDA, 1958). The research center is located in the Great Valley physiographic region, with mainly shale and dolomitic limestone as the primary parent materials for soils in this area (USDA, 1958). This area has a mean yearly temperature of 14.33°C and an average annual rainfall of 106 cm (USDA, 1958).

The predominant soil type in 2007 was the Nolichucky loam soil series. The site that the test was on is Nolichucky loam, undulating phase with two to five percent slopes (USDA, 1958). The soil is usually found on terraces and is formed from alluvium (USDA, 1958). It is usually low in respect to fertility and considered a good soil to support tobacco growth with high fertilization rates.

In 2008 the predominant soil series was the Ooltewah silt loam, commonly referred to as Lindsides silt loam. Ooltewah is formed from alluvium derived from limestone and contains a medium amount of organic matter. This soil type occurs in drainageways, sinkholes, and depressions. Most of the land classified as this soil type is in crops because of its naturally high fertility levels (USDA, 1958).

Experimental site and soil description at Springfield (SP)

Springfield is located in Robertson County, TN, which is in north central Tennessee. Springfield is just north of Nashville, TN and sits in the center of the Highland Rim physiographic province of TN. Agriculture is the predominant land use in this province, with tobacco being historically the major cash crop. The average temperatures are 14.4°C, with warm summers and mild winters. Average annual precipitation is 122 cm. The soils of the Highland Rim are loess capped and limestone derived (USDA, 1968).

In 2007 the predominant soil type was Mountview silt loam on 2-5 percent slopes. This soil is a deep well drained series and has a loess cap that is 0.61 to 0.91 meters. Mountview is better drained than Dickson soils which are commonly found adjacent to one another. This soil is

low in natural fertility and can be extremely acidic if not limed. All crops grown in Robertson county are well suited for this soil(USDA-NRCS, 2008; USDA, 1968).

In 2008 the test predominant soil type was Dickson silt loam on 2-5 percent slopes. These soils are moderately well drained and often have fragipans. The soil layers above the fragipan are easily worked and are permeable to roots and water. This soil is also low in natural fertility and can be very acidic if not limed. Dickson soils are predominately used for agricultural production(USDA-NRCS, 2008; USDA, 1968).

Experimental site and soil description at Lexington (LE)

The experimental site for LE was at Spindletop farm, owned by the University of Kentucky. The farm falls within the Bluegrass region in Kentucky (Newell, 2001). The name for this region was derived from Kentucky bluegrass, a cool season grass found in pastures in this region. However, Kentucky bluegrass is not native to Kentucky. The dominant soil series for both years at Spindletop was Maury silt loam. This soil series is classified as a fine, mixed, semiactive, Typic Paleidalf. Maury silt loams are upland soils and are well drained. The area has an average annual temperature of 12.2°C and mean annual precipitation of 114.3 cm. The predominant land use where this soil is found is agriculture, mostly cleared and used as cropland or hay-land (USDA-NRCS, 2008).

Experimental site and soil description at Dixon Springs (DS)

The Dixon Springs location was at the Dixon Springs Agricultural Center owned by the University of Illinois. It is the largest agricultural experiment station of its kind east of the Mississippi River. It is located in southern Illinois (Dixon Springs, 2009). The predominant soil

type for the field the study was conducted on was Grantsburg silt loam. This soil is capped with loess overlying sandstone, siltstone, and shale and has a tendency to develop fragipans. It is a moderately well drained soil found in areas with mean annual temperature of 12.8°C and mean annual precipitation of 111.8 cm (USDA-NRCS, 2008). This soil is classified as a fine-silty, mixed, active, mesic Oxyaquic Fragiudalf. These soils are mostly used for row crops, such as corn, wheat, or soybeans(USDA-NRCS, 2008).

Experimental site and soil description at Glade Spring (GL)

Glade Springs is in Washington County, VA, which is just northeast of Bristol, VA. This site is in the Ridge and Valley physiographic province in southwest Virginia. The ridge and valley province is characterized by shale or sandstone ridges with limestone valleys that run parallel to each other. The mean average temperature in January is 1°C and in July is 23°C. Mean annual precipitation is 114.3 cm (Washington County).

In both 2007 and 2008 the predominant soil type at GL was Frederick silt loam series. These soil series are derived from limestone and can be found on level to severe sideslopes, up to 60% slope. They are well drained soils and are classified as fine, mixed, semiactive Typic Paleudults. The level sites where this soil exists are cleared and cultivated for crops, where steeper areas are kept in pasture (USDA-NRCS, 2008).

Experimental Procedure 2007

A randomized complete block design with four blocks or replications was used at each site. In 2007, the treatments at Lexington, Greeneville, Springfield, and Dixon Springs applied in a five by two factorial treatment design. Glade Spring treatments were applied as a split-plot

treatment design in 2007. All nitrogen applied in this study was in the form of ammonium nitrate. Nitrogen was applied in five preplant rates and two sidedress rates. The five preplant nitrogen fertilization rates were 0, 67.4, 135, 202, and 270 kg/ha. Each preplant nitrogen rate received two sidedress nitrogen rates of 0 and 67 kg/ha. The sidedress nitrogen rates were applied to individual plots three weeks after transplanting. The ten treatment combinations can be found in Table 3.1. All plots at each location, except for Glade Spring in 2008, were four rows wide, 7.6 to 12.2 m in length and 1067mm in width. Glade Spring had two row plots in 2008. Plant populations at each site were 17,573 plants per hectare. See tables 3.2 and 3.3 for transplant and harvest dates. Phosphorus and potassium were applied according to Tennessee's burley tobacco production guide and based on soil samples collected before transplanting. All other production practices were also kept standard to guarantee that the only variation introduced into the experiment was nitrogen fertilization. All locations used LC (Jack and Bush, 2007) varieties in 2007; this is standard production practice in burley tobacco production. LC seed have been screened in order to remove high nornicotine converters so that nitrosonornicotine formation is kept at minimum levels during the curing process (Jack and Bush, 2007). KT 204LC was the variety used at all locations in 2007. The middle two rows from each four row plot were harvested when the plants were mature and air cured in conventional barns that were three to four tiers high. Once the curing process was finished the tobacco was stripped and separated into four stalk positions which is standard practice in burley tobacco production. Each plot was then graded using USDA grades (USDA-AMS, 1986) and the grades were converted into a grade index (Bowman et al., 1989) to be analyzed statistically. Once stripped and graded the cured tobacco was weighed and yield was calculated as kilograms per hectare. Twenty-five leaves were collected from each plot. These leaves were the fourth leaf from the top of the plant and

Table 3.1: 2007 Nitrogen Treatments

Treatments	Preplant Nitrogen Rate	Sidedress Nitrogen Rate
	kg/ha	kg/ha
1	0	0
2	0	67
3	67	0
4	67	67
5	135	0
6	135	67
7	202	0
8	202	67
9	270	0
10	270	67

Table 3.2: Transplant Dates

Year	Locations				
	GR	SP	GL	LE	DS
2007	5/22	5/24	5/22	-	6/13
2008	5/27	6/4	6/6	-	-

Table 3.3: Harvest Dates

Year	Locations				
	GR	SP	GL	LE	DS
2007	9/1	8/22	9/6	-	9/5-9/6
2008	9/3	9/9	9/25	-	-

were in the leaf stalk position (B grade) to conduct further test for TSNA content. Samples were sent to the University of Kentucky for TSNA analysis using the procedure described by Risner et al. (2001). TSNA data is reported for 2007. 2008 TSNA data is pending at this time.

Experimental Procedure 2008

Production practices remained the same in 2008 as in 2007 in regards to plant population, row widths, row length, and standard burley production methods. The Dixon Springs, IL location in 2007 was dropped from the study in 2008. For 2008 two nitrogen rates, treatments 9 and 10 (Table 3.1), were dropped from the study. Three varieties (TN 90LC, TN 90 high converter, and NC 7) were added to the test in addition to KT 204LC. TN 90LC received nitrogen treatments 1 through 8, while TN 90 high converter and NC 7 only received nitrogen treatments equivalent to treatments 1, 2, 4, 6, and 8 for 2007. A split-plot design with a factorial treatment arrangement in the whole plot was used in 2008. Nitrogen rates were split into the four preplant nitrogen fertilization rates and two sidedress rates resulting in a total of eight nitrogen treatments in the whole plots. Treatments used in 2008 can be found in Table 3.4. Plant sampling was also changed in 2008 and these changes will be covered in Plant Sampling and Analysis section later in this chapter.

Rotation Information

On the research centers for the universities a three year rotation is kept for all sites. For GR, LE, and DS the rotation is tobacco followed by two years of grass sod. Typically the sod is a mixture of grass species that can be utilized as hay for any livestock on the farm. At GR this mixture was timothy and orchard grass for the two years prior to the 2008 study. All sods on

Table 3.4: 2008 Nitrogen x Variety Treatments

Treatments	Variety	Preplant Nitrogen Rate	Sidedress Nitrogen Rate
		kg/ha	kg/ha
1	KT 204 LC	0	0
2	KT 204 LC	0	67
3	KT 204 LC	67	0
4	KT 204 LC	67	67
5	KT 204 LC	135	0
6	KT 204 LC	135	67
7	KT 204 LC	202	0
8	KT 204 LC	202	67
9	TN 90 LC	0	0
10	TN 90 LC	0	67
11	TN 90 LC	67	0
12	TN 90 LC	67	67
13	TN 90 LC	135	0
14	TN 90 LC	135	67
15	TN 90 LC	202	0
16	TN 90 LC	202	67
17	TN 90 HC	0	0
18	TN 90 HC	0	67
19	TN 90 HC	67	67
20	TN 90 HC	135	67
21	TN 90 HC	202	67
22	NC 7	0	0
23	NC 7	0	67
24	NC 7	67	67
25	NC 7	135	67
26	NC 7	202	67

university farms, except at GL, are managed by the extension recommendations for that crop. LE in 2008 was tobacco after tobacco rotation. At HR the rotation is two years of winter wheat followed by double-cropped soybeans, then one year of tobacco. Winter wheat and soybeans are also grown using university recommendations unless they are part of a study, then alternative practices may be utilized. GL leaves the field fallow for two years before rotating back into tobacco on the third year. Generally the cover is a mixture of weeds, volunteer fescue and clover.

Soil Sampling and Analysis

Soil nitrate (PSNT) samples were taken immediately before sidedress nitrogen treatments were applied, three weeks after transplanting. Samples were taken using standard soil probes, approximately 1.9 cm in diameter. Eight cores from the middle two rows, used harvest rows, were taken from each plot at a depth of 0-15 cm. Cores were placed in a bucket and mixed thoroughly, then transferred to plastic-lined paper bags for transport. Once in the laboratory the bags were opened and air-dried immediately. Samples were ground with a mortar and pestle to pass through a 2 mm sieve. Ground samples were then boxed and sent to the University of Tennessee Soil Testing Laboratory in Nashville, Tennessee. Nitrate measurements were taken from the soil samples using a nitrate sensitive electrode, in a pre-sidedress nitrate test, summarized by Donohue (1992). Soil samples were collected from all plots in 2007. Soil samples were only collected for KT 204LC and TN 90LC plots in 2008.

Plant Sampling and Analysis

Cardy® Nitrate Meter

Plant sap nitrate-N levels were measured using the Cardy® Nitrate meter manufactured

by Minolta. Calibration of the meter followed the methods described in the owner's manual for the meter (Cardy nitrate meter). Petiole nitrate samples were taken at sidedress, topping, and harvest in 2007. An additional sampling date was added in 2008 at five weeks after transplant (5WAT). Only KT 204LC and TN 90LC were sampled at sidedress, 5WAT, and topping in 2008. KT 204LC, TN 90LC and TN 90HC were sampled at harvest in 2008. Sampling dates for petiole nitrate in 2007 and 2008 can be found in Table 3.5. To measure petiole nitrate, four leaves were taken per plot. These leaves were the fully unfurled leaf closest to the apical bud, approximately 10-15 cm in length. At the harvest sampling interval the top leaf was taken for sampling. Leaf lamina was then stripped away from the base of the midrib, the petiole, for a length of 4 cm. This section of midrib was separated from the remainder of the leaf and stored in a plastic bag on ice for no longer than eight hours. Petioles were allowed to warm to room temperature before sap was extracted. Two petioles were crushed at once using a garlic press and the sap collected was placed on calibrated meter electrodes, giving two samples per plot. The meter was allowed to stabilize before nitrate measurements were recorded. In 2008, plastic syringes were used to extract sap instead of the garlic press. After each reading the meter was double rinsed with distilled water and blotted dry with a paper towel. The meter was recalibrated after each replication at the different locations to ensure accurate data.

CM 1000 Chlorophyll Meter® and other chlorophyll meters

Light reflectance measurements were taken using Spectrum's CM 1000 chlorophyll meter at the GR, GL, and HR locations. The CM 1000 measures light at 700 nm and 840 nm to give a relative chlorophyll index value (Fieldsout). Five chlorophyll measurements were taken per plot with the CM 1000 at GR, GL, and HR locations in both 2007 and 2008. These

Table 3.5: Presidedress Soil and Plant Sampling Dates

Year	Locations				
Sampling Interval	GR	SP	GL	LE	DS
2007					
Sidedress	6/18	6/12	6/19		7/6-7/9
Topping	7/31	7/24	7/30		8/17-8/21
Harvest	8/28	9/4	8/29		-
2008					
Sidedress	6/17	6/24	7/1		-
5WAT	6/30	7/11	7/14		-
Topping	8/4	8/7	8/11		-
Harvest	9/4	9/6	9/5		-

measurements were taken on the full most unfurled leaf closest to the apical bud and the same size as leaves sampled for the Cardy® Meter. Measurements were taken half way up the midrib and half way between the leaf margin and midrib. The chlorophyll meter was kept at a 90 degree angle to the leaf surface and the sun was at the back of the person taking the reading as described by Murdock (2004) in order to maximize reflected light and consistency. Chlorophyll samples were measured between 10 am and 4 pm as described by Murdock (2004). The meter was 30.5-38 cm away from the leaf surface in order to measure an area of leaf surface approximately 13-25 mm in diameter. The top leaf on the plant was measured at harvest in 2007 for chlorophyll analysis. In 2008, no harvest interval samples were taken with the chlorophyll meter at GR, GL, and HR locations due to the use of maleic hydrazide (trade name MH-30) at topping to control sucker growth. The top of the tobacco plant yellows as a result of using this chemical. All sampling dates can be found in table 3.5. At the DS location the SPAD 502® chlorophyll meter was used along with the Greenseeker® chlorophyll meter.

Statistical Methods and Analysis

The Mixed Model analysis of variance (ANOVA) in Statistical Analysis Systems (SAS 9.2) was used to determine differences between nitrogen treatments in 2007 or nitrogen by variety treatments in 2008 for yield, TSNA content, PSNT, petiole nitrate concentrations and chlorophyll measurements. Treatment effects were considered significant at alpha equals 0.1 level of probability. Mean separation was done using the Tukey-Kramer method at an alpha level of 0.1. Regression analyses were conducted for the simple linear and quadratic models using linear regression and polynomial regression analysis in SAS 9.2. Models and their respective parameters were considered significant at the alpha equals 0.1 level of probability. Regression analyses were conducted looking at the relationship between petiole nitrate, chlorophyll index levels, total nitrogen applied and PSNT levels versus absolute yield for individual locations. Petiole nitrate concentrations at harvest and total nitrogen were analyzed using the same regression models to evaluate their ability to predict total TSNA content. For total N and PSNT versus relative yield for the combined analyses, the quadratic plateau model in the PROC NLIN procedure in SAS 9.2 was used to find critical PSNT and total nitrogen levels. The quadratic plateau model was also used for total nitrogen versus total TSNA content relationship.

Chapter 4

Results and Discussion

2007 Yields

Yields were available from five locations in 2007. Yields for all locations in 2007 are reported in Table 4.1 along with mean separation analysis information for each nitrogen treatment. The main treatment effects means are in Table 4.2 along with the respective mean separation. Yields at GR, GL and LE were within the same range, while SP yields were the lowest of all locations. SP yields were depressed because of the drought in 2007. Weather data for all sites can be found in the appendix (Tables A.3-A.5). DS yields fall between SP and the other three locations. Yields for GR, GL, and LE exceeded Tennessee state burley tobacco historical averages for all nitrogen treatments in 2007.

Greeneville (GR)

In 2007, Tennessee experienced a severe drought, but the yields at GR did not indicate a yield reduction. Weather data from the experiment, Table A.3, show that in July of 2007 the station received a couple of timely rainfall events that allowed the crop to reach the yield levels reported. Yields for GR can be found in Table 4.1 and ranged from 3,578 kg/ha at the lowest nitrogen rate to 4,035 kg/ha at the highest nitrogen rate. The means for individual preplant by sidedress treatments did not differ significantly at GR. However, preplant nitrogen rates did differ significantly ($P=0.0288$) at GR and means are reported in Table 4.2. Yields for preplant nitrogen treatments increased with increasing nitrogen fertilization and ranged from 3,664 kg/ha to 4,034 kg/ha. Only the 0 kg N/ha preplant rate had yields that were statistically different from the maximum yield at the highest N rate. Sidedress rates at GR did

Table 4.1: Yields at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	LE	DS
(kg/ha)	(kg/ha)	-----Yield (kg/ha) -----				
0	0	3578	1876	3413 b*	3681	2547
0	67	3750	2040	3914 ab	3790	2875
67	0	3690	2037	3679 ab	3741	2815
67	67	3937	1997	4012 ab	4148	2997
135	0	3894	2134	4055 ab	4148	2955
135	67	3860	2397	4080 ab	3890	2841
202	0	3892	2193	3920 ab	4011	2923
202	67	4008	2189	3962 ab	4036	2968
270	0	4033	1858	3992 ab	4113	2876
270	67	4035	2020	4144 a	4134	3112

* Values with the same letters are not significantly different within the same location at 0.1 level of probability

Table 4.2: 2007 Yields and mean separations for the main treatment effects

Preplant N	Sidedress N	GR	SP	GL	LE	DS
(kg/ha)	(kg/ha)	----- Yield (kg/ha) -----				
0	-	3664 b*	1958	3664	3734 b	2711
67	-	3814 ab	2017	3845	3944 ab	2906
135	-	3877 ab	2265	4067	4019 ab	2898
202	-	3950 ab	2191	3891	4024 ab	2945
270	-	4034 a	1939	4068	4123 a	2994
-	0	3818 a	2020	3812 b	3939	2823
-	67	3918 a	2128	4002 a	4000	2959

* Values with the same letters are not significantly different within the same location at 0.1 level of probability

not differ statistically over all preplant rates ($P=0.1661$). Sidedress rate yields in Table 4.2 do show that yields were increased by 100 kg/ha though this increase was not significant. There was no interaction effect between preplant and sidedress nitrogen rates at GR in 2007.

Springfield (SP)

Yields at SP were the lowest of any site in 2007 due to the severe drought. Unlike GR, SP did not receive the timely rainfall in July (Table A.5). Because of limited rainfall, yields for this location do not show a clear pattern in their response to nitrogen fertilization. Individual nitrogen treatments did not differ significantly and yields ranged from 1,858 kg/ha in the 270 kg N/ha preplant + 0 kg N/ha sidedress treatment to 2,397 kg/ha in the 135 kg N/ha preplant + 67 kg N/ha sidedress treatment. Neither preplant or sidedress rates differed significantly with p-values of $P= 0.4460$ and $P= 0.4206$ respectively. No preplant N with sidedress N interaction effect was seen at this site ($P= 0.9459$). The drought at SP caused the yield response to vary greatly across nitrogen treatments (Table A.5).

Glade Springs (GL)

Yields at GL, Table 4.1, ranged from 3413 kg/ha in the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment to 4144 kg/ha in the 270 kg N/ha preplant + 67 kg N/ha sidedressed treatment. Only the lowest N rate gave yields significantly lower than the maximum yield at the highest N rate. Statistically, yields were maximized at this site with 67 kg N/ha either applied at preplant or sidedress. Preplant rates differed significantly ($P= 0.0996$) and ranged from 3,664 kg/ha at 0 kg N/ha to 4,068 kg/ha with 270 kg N/ha preplant. Tukey's mean separation did not find the means to be significantly different in the analysis, but the main effect of preplant treatments was significant at $\alpha = 0.1$. Sidedress treatments across all preplant nitrogen

treatments were also significant ($P = 0.0667$). The 67 kg N/ha sidedressed plots yielded 4,002 kg/ha to 3,812 kg/ha for the 0 kg N/ha sidedressed plots. No preplant N with sidedress N interaction effect was observed at GL indicating that all preplant rates responded in a similar fashion to sidedress nitrogen applications. Overall yield at GL increased with increasing nitrogen fertilizer application.

Lexington (LE)

Yields at LE (Table 4.1 and 4.2) were similar to GR and GL in 2007. Individual treatment means ranged from 3,681 kg/ha in the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment to 4,148 kg/ha in both the 135 kg N/ha + 0 kg N/ha and 67 kg N/ha + 67 kg N/ha nitrogen treatments. Yield for preplant nitrogen ranged from 3,734 kg/ha in the 0 kg N/ha preplant treatment to 4,123 kg/ha in the 270 kg N/ha preplant treatment. Only the yield for lowest preplant nitrogen rate differed from the yield for the highest preplant rate. Yields were statistically maximized with a total of 67 kg N/ha applied prior to transplant. There was no difference across preplant nitrogen rates when sidedress nitrogen was applied ($P = 0.5184$). No interaction effect was observed between preplant and sidedress nitrogen treatments ($P = 0.2881$). Though the yields between individual nitrogen treatments were not statistically significant, an increase of 467 kg/ha from the lowest yielding treatment to the highest yielding treatment would be considered significant from the financial perspective of a burley tobacco producer.

Dixon Springs (DS)

Yields at DS are more closely related to typical burley yields achieved on commercial farms. The individual treatment and main effect treatment means can be found in Tables 4.1 and 4.2. Individual treatment yields at DS ranged from 2547 kg/ha at the lowest nitrogen rate to

3,112 kg/ha for the highest nitrogen rate applied. Yields among the individual nitrogen treatments were not significantly different. Yields within each preplant nitrogen rate did not differ from each other ($P = 0.2633$). Sidedress nitrogen rates also were not significantly different ($P = 0.1081$) over all preplant nitrogen rates. All preplant and sidedress combinations behaved similarly at this location resulting in no interaction effect ($P = 0.4774$).

2008 Yields

In 2008 yields were reported at GR, SP, and LE. Yields at GL and DS are not reported in 2008. Since there were four varieties in the test, two which received all nitrogen treatments and two that only received five of the nitrogen treatments, two separate analyses of variance were conducted, one for the two varieties receiving all nitrogen treatments, and the second for all varieties over five common nitrogen rates. Table 4.3 gives the mean yields for KT 204LC and TN 90LC, which received all nitrogen treatments at all locations in 2008. Table 4.4 gives the main treatment means for KT 204LC and TN 90LC at all locations in 2008. In 2008, all sites had more rainfall than 2007 and responses to nitrogen fertilization were more clearly seen. No sites were substantially higher yielding than the other sites, in 2008.

Greeneville (GR)

Yields in 2008 (Table 4.3) at GR were not as high as in 2007. This may be partly due to disease pressure. GR experienced disease pressure from *Phytophthora parasitica* var. *nicotiana*, the organism commonly referred to as black shank. Even though the field used was in a three year rotation with grass sod, some plots were seriously impaired by black shank. Plots were rated for black shank during the growing season and based on the number of plants harvested, selected plots deleted for yield analysis. The threshold set for deleting plots was a 60% stand or

Table 4.3: Yields for KT 204LC and TN 90LC for all nitrogen treatments at all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	LE	GL
	(kg/ha)	(kg/ha)	----- Yield (kg/ha)-----			
KT 204LC	0	0	2356	2104 c*	2153 de	-**
KT 204LC	0	67	2686	2551 abc	2459 bcde	-
KT 204LC	67	0	2367	2672 abc	2619 abcde	-
KT 204LC	67	67	2617	2932 ab	2696 abcd	-
KT 204LC	135	0	2386	2825 ab	2823 abcd	-
KT 204LC	135	67	2527	2875 ab	2946 abc	-
KT 204LC	202	0	2633	2842 ab	3091 ab	-
KT 204LC	202	67	2649	3090 a	3166 a	-
TN 90LC	0	0	2184	2089 c	2006 e	-
TN 90LC	0	67	2646	2352 bc	2369 cde	-
TN 90LC	67	0	2179	2405 bc	2332 cde	-
TN 90LC	67	67	2377	2736 abc	2605 abcde	-
TN 90LC	135	0	2391	2623 abc	2491 abcde	-
TN 90LC	135	67	2417	2510 abc	2903 abc	-
TN 90LC	202	0	2367	2621 abc	2931 abc	-
TN 90LC	202	67	2599	2902 ab	3100 ab	-

* Values with the same letters are not significantly different within the same location at 0.1 level of probability

** Data on yield for GL has not been reported

Table 4.4: Main treatment effect means for Yield of KT 204LC and TN 90LC at all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	LE	GL
	(kg/ha)	(kg/ha)	-----Yield (kg/ha)-----			
-	0	-	2468	2274 b*	2247 c	-**
-	67	-	2385	2686 a	2563 b	-
-	135	-	2430	2708 a	2791 ab	-
-	202	-	2562	2864 a	3072 a	-
-	-	0	2358 b	2523 b	2556 b	-
-	-	67	2565 a	2743 a	2780 a	-
KT 204LC	-	-	2527 a	2736 a	2744 a	-
TN 90LC	-	-	2395 b	2530 b	2592 b	-

* Values with the same letters are not significantly different within the same location at 0.1 level of probability

** Data on yield for GL has not been reported

less at harvest based on a plant population of 17,573 plants per hectare. Black shank pressure is important in this study because KT 204LC has a higher resistance rating to black shank than TN 90LC.

For individual treatments at GR in 2008 no yield difference was found. Yields ranged from 2,179 kg/ha for TN 90LC at the 67 kg N/ha preplant + 0 kg N/ha sidedressed to 2,686 kg/ha for KT 204LC at the 0 kg N/ha preplant + 67 kg N/ha sidedressed treatments. Though not statistically significant, there was an increase in yields for both varieties as nitrogen rate increased. Means for preplant, sidedress, and varieties can be found in Table 4.4. Preplant treatments were not significantly different in regard to yield ($P = 0.2644$). Sidedress treatments did produce a difference ($P = 0.0042$) in yield, with 0 kg N/ha sidedressed plots yielding 2,358 kg/ha compared to 2,565 kg/ha for plots that were sidedressed with 67 kg N/ha. Variety differences were seen at GR ($P = 0.0451$) with KT204LC having a mean yield of 2,527 kg/ha and TN 90LC yielding 2,395 kg/ha. Both varieties responded similarly to sidedress and preplant nitrogen rates. Differences in varieties may partially be due to differing black shank resistance.

Springfield (SP)

At SP in 2008, yields ranged from 2,089 kg/ha for TN 90LC receiving 0 kg N/ha preplant + 0 kg N/ha sidedress to 3,090 kg/ha for KT 204LC at the 202kg N/ha preplant + 67 kg N/ha sidedress treatment (Table 4.3). Maximum yields were reached at the 0 kg N/ha preplant + 67 kg N/ha sidedress treatment in KT 204LC and TN90LC. In both varieties the lowest nitrogen treatment produced the lowest yield. Yields were statistically different between the lowest and highest nitrogen treatments in both varieties. Preplant rates at SP were significantly different ($P = 0.001$) and means for preplant rates over all sidedress and variety treatments ranged from 2,274

kg/ha for the 0 kg N/ha rate to 2,864 kg/ha for the 202 kg N/ha rate (Table 4.4). Only the 0 kg N/ha preplant rate was different from all other preplant rates. Yields were statistically optimized at the 67 kg N/ha preplant rate. Sidedress rates also differed ($P = 0.0051$) when compared over all preplant and variety combinations. Means for the two sidedress rates were 2,523 kg/ha for 0 kg N/ha and 2,743 kg/ha for 67 kg N/ha. KT 204LC produced higher yields than TN 90LC at SP in 2008. Means for the variety effects can be found in Table 4.4 and the overall effect was highly significant with $P = 0.0016$. No interaction effects were seen between all other treatment combinations at SP. Yield at this site increased numerically as nitrogen rate increased up to 135 total kg N/ha, while above this level no further yield increase occurred.

Lexington (LE)

LE saw more response to nitrogen fertilization in 2008 than any of the sites over the two year study. Yields ranged from 2,006 kg/ha at 0 kg N/ha preplant + 0 kg N/ha sidedress for TN 90LC to 3,166 kg/ha at 270 kg N/ha preplant + 67 kg N/ha sidedress for KT 204LC. Individual treatment means can be found in Table 4.3. KT 204LC showed a response to sidedress at the 0 kg N/ha preplant rate, but there was no significant increase in yield for KT 204LC from 67 kg N/ha preplant to the highest nitrogen rate applied. However, TN 90LC did not maximize yields until a total nitrogen rate of 135 kg N/ha was applied at LE. There was a yield increase of 412 kg/ha for TN 90LC from the 135 kg N/ha preplant with no sidedress to the 135 kg N/ha sidedress treatments. Mean separation does not show this increase to be significant, but because of the value of burley tobacco this would be a significant increase to producers. Practical maximum yields were not obtained until a total nitrogen fertilization rate of 202 kg N/ha was applied at LE.

Table 4.4 shows the main treatment effects for LE in 2008. Preplant and sidedress main treatment effects were significant with $P < 0.0001$ and $P < 0.0180$, respectively. Preplant means ranged from 2,247 kg/ha at the 0 kg N/ha preplant rate to 3,072 kg/ha at the 202 kg N/ha preplant rate. The 0 and 67 kg N/ha preplant rates both differed from the 202 kg N/ha preplant rates. Sidedress nitrogen treatment yields were 2,556 kg/ha for the 0 kg N/ha rate and 2,780 kg/ha for the 67 kg N/ha rate. KT 204LC yielded 2,744 kg/ha compared to 2,592 kg/ha for TN 90LC at LE in 2008. This difference over all nitrogen rates was significant with $P < 0.0027$. In 2008, LE was the only site in the study that was a tobacco after tobacco rotation which may account for a greater response in yield to nitrogen fertilization.

All Locations Combined Yield for KT 204 LC

A combined analysis was conducted for the eight nitrogen treatments in common between 2007 and 2008. Yields for KT 204 LC were used from 2008, since this variety was used at all sites in 2007. The combined analysis for yield included eight of the nine experiments over the two years the study was conducted. Table 4.5 has the means for the eight individual nitrogen treatments as well as the preplant and sidedress main effect means. Preplant main effect means are in the far right column and sidedress main effect means can be found below individual treatment means in their respective columns. Over the two year study individual treatment means ranged from 2,713 kg/ha for the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment to 3,246 kg/ha for the 202 kg N/ha + 67 kg N/ha sidedress treatment. Over all sites maximum yields were reached statistically at the 67 kg N/ha preplant + 67 kg N/ha sidedress treatment. Above 135 kg total N/ha there was no statistical response to further nitrogen application was observed for either preplant or sidedress application. Preplant main effects means show the same response with

Table 4.5: Combined analysis for KT 204LC Yield at all locations in 2007 and 2008

Preplant N (kg/ha)	Sidedress N ----- (kg/ha)-----		Avg. (kg/ha)
	0	67	
0	2713 d**	3005 bc	2859 C*
67	2952 c	3168 ab	3060 B
135	3152 ab	3177 ab	3165 AB
202	3188 ab	3246 a	3217 A
Avg.	3002 B	3149 A	

*Means within main effects are not significantly different when followed by the same capital letter (p= 0.10).

**Means for the preplant by sidedress combinations are not significantly different when followed by the same lower case letter.

yields being maximized at the 135 kg N/ha rate. Preplant rates differed ($P < 0.0001$) with the 0 and 67 kg N/ha rates being significantly different from the 202 kg N/ha preplant rate. Sidedress rates also differed ($P < 0.0001$) with means of 3,002 kg/ha for the 0kg N/ha rate and 3,149 kg/ha for the 67 kg N/ha sidedress rate. There was an interaction between the preplant and sidedress rates when all sites were combined ($P = 0.0195$). The 0 and 67 kg N/ha preplant saw significant yield increases when sidedress nitrogen was applied, whereas the 135 and 202 kg N/ha preplant rates did not get a significant increase in yield when sidedress nitrogen was applied. The combined analysis shows that maximum yields for KT 204LC are reached when a total of 135 kg N/ha are applied.

Yield Analysis for all varieties grown in 2008 over five nitrogen rates

In 2008, NC 7 and a TN 90 high converter (HC) line were incorporated into the study with TN90LC and KT 204LC. NC 7 and TN 90HC only received five of the nitrogen treatments,

so the following analysis uses total nitrogen instead of a preplant and sidedress rates. The varieties were selected to give an overall representation of the genotypes found in the current burley tobacco market and to compare low converter (LC) lines to a line that has not undergone the LC screening process. Yields for the four varieties across all nitrogen rates at GR, SP and LE can be found in Table 4.6 and the yields for the varieties for all locations combined can be found in Table 4.7. Main effects for individual locations are also in Table. 4.6, while the main effects for the combined analysis are in Table 4.7.

Greeneville (GR)

At GR yields ranged from 1,967 kg/ha for TN 90HC at the 0 kg N/ha rate to 2,647 kg/ha for KT 204LC at 270 kg N/ha rate. TN 90HC with 0 kg N/ha was the only individual treatment that differed from the highest yielding variety by nitrogen rate combination. Across all nitrogen treatments NC 7 and KT 204LC consistently produced higher yields than TN 90LC and TN 90HC. Yields for total nitrogen rates differed ($P= 0.0087$) when averaged across all varieties. Yields for total nitrogen were statistically maximized at the 67 kg N/ha rate, with yields ranging from 2,220kg/ha at 0 kg N/ha to 2,608 kg/ha for the 270 kg N/ha rate. Varieties also differed ($P= 0.0045$) in yields when averaged across all nitrogen rates at GR. TN 90HC was the lowest yielding variety with 2,318 kg/ha and KT 204LC was the highest yielding variety with 2,565 kg/ha. NC 7 was the second highest yielding variety followed by TN 90LC. There was no interaction effect between nitrogen rates and variety at GR.

Springfield (SP)

SP showed more response to nitrogen fertilization across all varieties than GR in 2008. Yields ranged from 1,927 kg/ha for TN 90HC with 0 kg N/ha to 3,088 kg/ha for KT 204LC with

Table 4.6: Yields by Total Nitrogen for all varieties in 2008

Location	Variety	Total Nitrogen Applied					Variety
		0	67	135	202	270	Avg. Yield
----- Yield (kg/ha) -----							(kg/ha)
GR	KT 204LC	2355 ab	2684 a	2615 a	2525 ab	2647 a	2565 A*
	TN 90LC	2182 ab	2633 a	2384 ab	2423 ab	2597 a	2444 AB
	TN 90HC	1967 b	2309 ab	2349 ab	2464 ab	2499 ab	2318 B
	NC 7	2375 ab	2585 a	2574 a	2550 ab	2597 a	2554 A
	Avg. Yield	2220 B	2553 A	2480 A	2490 A	2608 A	
SP	KT 204LC	2103 de	2550 abcde	2929 abc	2872 abc	3088 a	2708 A
	TN 90LC	2120 de	2350 bcde	2734 abcd	2508 abcde	2900 abc	2552 B
	TN 90HC	1927 e	2328 cde	2545 abcde	2734 abcd	2848 abc	2476 B
	NC 7	2036 e	2448 abcde	2997 ab	3040 a	2981 abc	2700 A
	Avg. Yield	2046 C	2419 B	2801 A	2788 A	2954 A	
LE	KT 204LC	2153 hij	2459 defghij	2696 bcdefghi	2946 acdbef	3166 abc	2684 B
	TN 90LC	2006 ij	2369 efghij	2605 bcdefghij	2903 abcdefg	3100 abcd	2597 BC
	TN 90HC	1962 j	2221 ghijk	2253 fhijk	2805 bcdefgh	3001 abcde	2448 C
	NC 7	2234 ghijk	2508 cdefghij	2874 abcdefg	3289 ab	3511 a	2883 A
	Avg. Yield	2089 D	2389 CD	2607 BC	2986 AB	3194 A	

* Mean comparisons are within locations. Main effect means followed by the same capital letter are not significantly different at $p=0.10$. Variety by N rate means followed by the same lower case letter are not significantly different at $p=0.10$.

Table 4.7: Combined Analysis for all varieties and total nitrogen in 2008

Location	Variety	Total Nitrogen Applied					Variety
		0	67	135	202	270	Avg. Yield
----- Yield (kg/ha) -----							(kg/ha)
All Sites	KT 204LC	2204 hij	2564 bcdefgh	2747 abcde	2781 abcdef	2967 ab	2652 A*
	TN 90LC	2101 ij	2434 efghi	2589 bcdefgh	2614 bcdefgh	2866 abcd	2521 B
	TN 90HC	1952 j	2286 ghij	2382 fghi	2668 abcdefg	2783 abcdef	2414 C
	NC 7	2215 hij	2514 defghi	2815 abde	2959 abc	3060 a	2712 A
	Avg. Yield	2118 D	2449 C	2633 BC	2755 AB	2919 A	

* Main effect means followed by the same capital letter are not significantly different at $p=0.10$. Variety by N rate means followed by the same lower case letter are not significantly different at $p=0.10$

270 kg N/ha. No statistically significant yield response was seen for NC 7 and KT 204LC above the 67 kg N/ha rate. TN 90LC and TN 90HC did not show a yield response above 135 kg N/ha. Total nitrogen rates differed ($P < 0.0001$) and yields averaged over all varieties ranged from 2,046 kg/ha at the 0 kg N/ha rate to 2,954 kg/ha at the 270 kg N/ha rate. Yields were maximized at 135 kg N/ha across all varieties at SP in 2008. Varieties also differed ($P = 0.0029$) in yield levels. NC 7 and KT 204LC were both at 2700 kg/ha and TN 90HC produced the lowest yields with only 2,476 kg/ha. KT 204LC and NC 7 were significantly different from TN 90LC and TN 90HC in yield. No variety by total nitrogen rate interaction effect was seen meaning all varieties responded the same to increasing nitrogen fertilization.

Lexington (LE)

LE had the greatest range of yields for the variety by nitrogen rate part of the study with yield ranging from 1,962 kg/ha for TN 90HC with 0 kg N/ha to 3,511 kg/ha for NC 7 fertilized with 270 kg N/ha. NC 7 and TN 90LC had no yield response above 135 kg/ha, while KT 204LC saw no response above 67 kg N/ha. TN 90HC showed the most response to nitrogen fertilization with yield not maximizing until the 202 kg N/ha rate. Yields for total nitrogen rates differ ($P < 0.0001$) and range from 2,089 kg/ha for the 0 kg N/ha rate to 3,194 kg/ha for the 270 kg N/ha rate. A yield response is observed up to 202 kg N/ha, above which yields are not significantly different. Yields between varieties averaged over all nitrogen rates differ ($P < 0.0001$). TN 90HC produced the lowest yields, while NC 7 produced the highest yields at LE. KT 204LC was the second highest yielding variety follow by TN 90LC. Yield of NC 7 was significantly higher than all other varieties at this site. There was no interaction between varieties and total nitrogen applied.

Combined Analysis for Varieties by Total Nitrogen

Yields for the four varieties by nitrogen rate across all locations are found in Table 4.7. Yields averaged across all sites range from 1,952 kg/ha for TN 90HC at 0 kg N/ha to 3,060 kg/ha for NC 7 at 270 kg N/ha. KT 204LC did not show a statistically significant yield response to increased nitrogen above 67 kg N/ha, while the three other varieties show no response to increase nitrogen application above 135 kg N/ha. When averaged over all varieties, yields differed ($P < 0.0001$) over total nitrogen rates applied; yields were maximized with 202 kg N/ha. Yields among varieties differed ($P < 0.0001$) with NC 7 averaging the highest yield, 2,712 kg/ha and TN 90LC averaging the lowest yield, 2,414 kg/ha. KT 204LC yield did not differ from NC 7, but TN 90LC did differ. KT 204LC and NC 7 consistently produced higher yields than TN 90LC and TN 90HC over all nitrogen rates. KT 204LC maximized yields at a 67 kg N/ha at every location except LE while the other varieties maximized yields at 135 kg N/ha over all sites.

2007 Tobacco Specific Nitrosamines (TSNA)

TSNA data is only reported for GR, SP, GL, and LE for 2007. DS data for TSNA accumulation was not available for the completion of this thesis. 2008 TSNA analysis for GR, SP, GL, and LE has not yet been compiled. However, samples of the cured leaf are currently at the University of Kentucky and awaiting analysis to determine TSNA content. The following data is from the sites that have reported their TSNA data. Regression models will be reported later in the chapter to show any relationship that TSNA accumulation has with any of the in field monitoring that was done in 2007. GL TSNA levels are higher than the other sites in the study. Higher TSNA levels were also reported by Waynick (2007), where the higher levels of TSNA at GL were contributed to shielding of the barn to the prevailing wind resulting in the potential of

higher relative humidity during curing. Other research has shown that TSNA levels are affected by curing conditions and this is thought to be the situation in GL.

Greeneville (GR)

Data for total TSNA content and individual TSNA can be found in Table 4.8 for Greeneville in 2007. Table 4.8 contains leaf lamina TSNA content only; midrib TSNA concentrations were not measured in this study. Total TSNA content tended to increase with increasing nitrogen rates, but the 67 N kg/ha preplant and 67 N kg/ha sidedress treatment were statistically the same as the 240 N kg/ha preplant and 67 N kg/ha sidedress. Total TSNA content in leaf lamina ranged from 0.325 µg/g to 1.440 µg/g. The only rates significantly lower than the 240 N kg/ha preplant and 67 N kg/ha sidedressed were the three lowest nitrogen rates at GR. in Preplant nitrogen rates significantly affected TSNA content ($P < 0.0001$) in cured burley tobacco leaf at GR in 2007. Sidedress nitrogen rates at GR also affected ($P = 0.0089$) total TSNA content in the cured leaf. However, no interaction effect was seen between the preplant and sidedress N rates ($P = 0.9957$). Table 4.8 shows that when sidedress N was applied the total TSNA

Table 4.8: 2007 TSNA for GR

Preplant N (kg/ha)	Sidedress N (kg/ha)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	NNK (µg/g)	Total TSNA (µg/g)
0	0	0.093 d*	0.223 d	0.000	0.008	0.325 d
0	67	0.145 cd	0.385 cd	0.009	0.021	0.563 cd
67	0	0.273 bcd	0.460 bcd	0.008	0.023	0.765 bcd
67	67	0.405 abc	0.583 abc	0.010	0.029	1.025 abc
135	0	0.308 abcd	0.603 abc	0.018	0.016	0.940 abc
135	67	0.483 ab	0.710 abc	0.022	0.000	1.220 abc
202	0	0.313 abcd	0.675 abc	0.008	0.009	1.008 abc
202	67	0.428 abc	0.783 ab	0.000	0.030	1.243 ab
270	0	0.420 abc	0.833 a	0.004	0.016	1.268 ab
270	67	0.568 a	0.825 a	0.022	0.027	1.440 a

* Values with the same letters are not significantly different at 0.1 level of probability

content increased within leaf lamina for each preplant N rate.

Among the individual TSNA, NAT had the highest concentrations in the cured leaf, followed by NNN with NAB and NNK being of little importance to total TSNA accumulation. NAT and NNN concentrations increased with increasing nitrogen rates and with sidedressing, but at the same total N rate sidedressed and non-sidedressed treatments generally had about the same TSNA content. For NNN, preplant nitrogen differed significantly ($P < 0.0001$) as did sidedress nitrogen rates ($P = 0.0052$). Preplant and sidedress nitrogen treatments were also significantly different for NAT accumulation with p-values of $P < 0.0001$ and $P = 0.0566$ respectively. Preplant and sidedress rates were not significantly different for NAB and NNK. For all individual TSNA no interaction effects were observed at GR in 2007.

Springfield (SP)

Individual and total TSNA content for cured leaf at SP can be found in Table 4.9. Unlike GR total TSNA for individual treatments were not significantly different. Total TSNA content ranged from $0.980 \mu\text{g/g}$ to $1.508 \mu\text{g/g}$ in cured leaf lamina. The lower range was slightly higher than GR and LE sites, with a maximum nitrosamine level very similar to those two sites. There were significant differences ($P = 0.0644$) in mean total TSNA content between preplant nitrogen rates for this site. The 0 kg N/ha preplant had the lowest TSNA content in cured leaf lamina and was only significantly different from the 135 kg N/ha preplant rate at this site. Sidedress nitrogen rates did not differ statistically from non-sidedress rates at this site. No interaction effect between preplant and sidedress nitrogen rates was observed at SP.

Individual TSNA in cured leaf lamina did not differ statistically for each nitrogen treatment at SP. Preplant nitrogen treatments differed significantly for NNN with $P = 0.0135$. NNN

Table 4.9: 2007 TSNA for SP

Preplant N (kg/ha)	Sidedress N (kg/ha)	NNN ($\mu\text{g/g}$)	NAT ($\mu\text{g/g}$)	NAB ($\mu\text{g/g}$)	NNK ($\mu\text{g/g}$)	Total TSNA ($\mu\text{g/g}$)
0	0	0.355	0.595	0.009	0.018	0.980
0	67	0.380	0.575	0.055	0.043	1.053
67	0	0.568	0.583	0.000	0.000	1.150
67	67	0.585	0.780	0.026	0.016	1.405
135	0	0.705	0.750	0.020	0.034	1.508
135	67	0.643	0.750	0.052	0.038	1.483
202	0	0.483	0.675	0.011	0.002	1.170
202	67	0.648	0.663	0.017	0.021	1.343
270	0	0.508	0.688	0.035	0.037	1.270
270	67	0.538	0.693	0.017	0.029	1.275

followed the same trend as total TSNA content in that only the 0 kg N/ha differed from the 135 kg N/ha preplant rate. The 0 kg N/ha preplant had the lowest NNN accumulation at this site. There were significant differences ($P = 0.0915$) in sidedress nitrogen rates with respect to NAB. The 67 kg N/ha sidedressed had higher levels of NAB than the non-sidedressed treatments over all preplant rates, 0.033 $\mu\text{g/g}$ and 0.015 $\mu\text{g/g}$ respectively. No other significant effects were seen for any other individual TSNA.

Glade Spring (GL)

Table 4.10 shows the TSNA data for GL with mean separations by individual nitrogen treatments for 2007. GL had markedly higher TSNA accumulation than any of the other three sites presented in this research. This is thought to be because of barn location contributing to decreased air flow through the barn. Decreased air flow increases relative humidity and increases the duration of the yellowing phase during the curing process (Waynick, 2007; Burton et. al., 1989; Bush et al., 2001). Total TSNA content at GL increased with increasing nitrogen fertilization and ranged from 2.893 $\mu\text{g/g}$ to 5.478 $\mu\text{g/g}$ in leaf lamina. The 0 kg N/ha preplant +

Table 4.10: 2007 TSNA for GL

Preplant N (kg/ha)	Sidedress N (kg/ha)	NNN (µg/g)	NAT (µg/g)	NAB (µg/g)	NNK (µg/g)	Total TSNA (µg/g)
0	0	0.945 b*	1.678 c	0.063 ab	0.207	2.893 c
0	67	1.218 ab	2.538 abc	0.152 ab	0.261	4.180 abc
67	0	1.198 ab	2.070 bc	0.191 ab	0.193	3.650 bc
67	67	1.283 ab	2.433 bc	0.127 ab	0.357	4.192 abc
135	0	1.315 ab	2.405 abc	0.001 b	0.205	3.928 abc
135	67	1.413 ab	2.823 ab	0.214 a	0.245	4.695 ab
202	0	1.668 a	2.810 ab	0.189 ab	0.349	5.103 ab
202	67	1.283 ab	2.913 ab	0.131 ab	0.354	4.680 ab
270	0	1.313 ab	2.748 ab	0.175 ab	0.319	4.555 abc
270	67	1.598 ab	3.348 a	0.226 a	0.304	5.478 a

* Values with the same letters are not significantly different at 0.1 level of probability

0kg N/ha sidedress and 67 kg N/ha preplant + 0 kg N/ha sidedress were the only treatments that significantly differed from the highest TSNA concentration, which occurred in the 270 kg N/ha preplant + 67 kg N/ha sidedress treatment. Preplant and sidedress nitrogen treatments were found to be significantly different, $P = 0.0053$ and $P = 0.0173$ respectively. No interaction effect between sidedress and preplant treatments was observed. This can be seen in Table 4.10 as preplant rates that were sidedressed are higher in TSNA content than the same preplant rates that were not sidedressed. However, when looking at total nitrogen applied there was no effect on TSNA accumulation when sidedress applications were used instead of applying all nitrogen at preplant.

The individual TSNA follow the same trend as SP and GR in that NAT is found in the greatest concentration, followed by NNN, NNK and NAB. NAB seems to be higher at this location than at any of the other sites. NAB concentration does not show a definitive pattern in

response to nitrogen fertilization like NNN and NAT. However, the interaction effect of preplant+ sidedress was significant, $P=0.0388$. Preplant and sidedress treatments were highly significant with regards to NAT ($P<0.0007$ and $P=0.0019$). The 0+0, 67+0, and 67+67 kg N/ha preplant + sidedress treatments were significantly lower in regards to NAT than the 270 + 67 kg N/ha preplant + sidedress treatments. No other main treatment effects were significant at GL, but Tukey's mean separation procedure identified significant treatment differences for NNN and NAB. The NNN differences generally followed N rate differences, but the NAB differences came from one low value with no obvious explanation.

Lexington (LE)

LE TSNA content (Table 4.11) was in the same range as SP and GR. Total TSNA concentrations in cured leaf lamina ranged from 0.470 $\mu\text{g/g}$ in the 0 preplant + 0 sidedress kg N/ha to 1.717 $\mu\text{g/g}$ in the 202 preplant + 67 sidedress kg N/ha. The lowest nitrogen treatment was significantly different than the highest six nitrogen treatments at LE in regards to total TSNA content. TSNA concentrations

Table 4.11: 2007 TSNA for LE

Preplant N (kg/ha)	Sidedress N (kg/ha)	NNN ($\mu\text{g/g}$)	NAT ($\mu\text{g/g}$)	NAB ($\mu\text{g/g}$)	NNK ($\mu\text{g/g}$)	Total TSNA ($\mu\text{g/g}$)
0	0	0.161 c*	0.267 b	0.022	0.020	0.470 b
0	67	0.400 abc	0.616 ab	0.058	0.022	1.096 ab
67	0	0.318 bc	0.613 ab	0.053	0.039	1.022 ab
67	67	0.366 abc	0.682 ab	0.063	0.060	1.170 ab
135	0	0.573 ab	0.833 a	0.066	0.059	1.531 a
135	67	0.377 abc	0.814 a	0.038	0.058	1.287 a
202	0	0.538 abc	0.702 ab	0.075	0.048	1.363 a
202	67	0.751 a	0.832 a	0.073	0.060	1.717 a
270	0	0.368 abc	0.799 a	0.041	0.049	1.257 a
270	67	0.518 abc	0.890 a	0.055	0.034	1.496 a

* Values with different letters are significantly different at 0.1 level of probability

were significantly different ($P = 0.0011$) at LE, with higher preplant nitrogen rates having higher total TSNA content in leaf lamina. Applying sidedress nitrogen also significantly ($P = 0.0474$) increased total TSNA accumulation. No interaction effect was observed at LE and this continues the trend that no nitrogen treatment combinations preformed differently than the other treatments.

LE continued the same trend of NAT being the highest TSNA followed by NNN, then NAB and NNK being relatively low and about the same concentrations. NAT concentrations ranged from $0.267 \mu\text{g/g}$ to $0.890 \mu\text{g/g}$ with the lowest concentration in the zero nitrogen treatment and the highest NAT concentration in the 270 kg N/ha preplant plus 67 kg N/ha sidedressed treatment. Both preplant and sidedress treatments were found to be significantly different in regards to NAT with p-values of $P = 0.0039$ and $P = 0.0734$ respectively. Only preplant nitrogen treatments were found to be significantly different in NNN concentrations ($P = 0.0035$). No interaction effects were found at the LE location when analyzing individual TSNA.

All Locations Combined TSNA

TSNA content from the four locations in 2007 was combined and analysis of variance was conducted on NNN, NAT and total TSNA content. Table 4.12 shows the mean separation by N rate for NNN, NAT and total TSNA. Total TSNA content ranged from $1.1667 \mu\text{g/g}$ in the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment to $2.422 \mu\text{g/g}$ in the 270 kg N/ha preplant + 67 kg N/ha sidedress treatment. Above the 135 kg/ha total nitrogen applied no response in total TSNA content was observed as more nitrogen was applied. Preplant nitrogen treatments differed in total TSNA content ($P < 0.0001$). Sidedress nitrogen treatments also differed in total TSNA content ($P = 0.0005$). No interaction effect was observed in total TSNA content between preplant

Table 4.12: Combined TSNA for GR, SP, GL, and LE for 2007

Preplant N (kg/ha)	Sidedress N (kg/ha)	NNN (µg/g)	NAT (µg/g)	Total TSNA (µg/g)
0	0	0.388 c*	0.691 d	1.1667 d
0	67	0.537 bc	1.022 bc	1.716 bc
67	0	0.589 abc	0.931 cd	1.647 cd
67	67	0.655 ab	1.114 bc	1.941 abc
135	0	0.725 ab	1.148 abc	1.976 abc
135	67	0.718 ab	1.245 ab	2.124 abc
202	0	0.750 ab	1.216 abc	2.138 abc
202	67	0.777 a	1.297 ab	2.245 ab
270	0	0.652 ab	1.267 ab	2.087 abc
270	67	0.805 a	1.439 a	2.422 a

* Values with the same letters are not significantly different within columns at 0.1 level of probability

and sidedress rates and the data shows that for every preplant rate the sidedressing treatment increased total TSNA content by about the same amount. As nitrogen fertilization increased, total TSNA content tended to increase across all sites.

The main individual TSNA, NNN and NAT, follow the same trend as total TSNA content in that as nitrogen fertilization increases so does the concentration of the NNN and NAT in cured lamina. NNN is found in lower levels, ranging from 0.388 µg/g to 0.805 µg/g, than NAT which ranges from 0.691 µg/g to 1.439 µg/g. Preplant rates differ for NNN ($P=0.0001$) and NAT ($P=0.0001$) concentrations. As preplant nitrogen rates increase NNN and NAT concentrations also increase. Sidedress nitrogen treatments also increased the concentration of NNN ($P=0.0242$) and NAT ($P=0.0003$) in cured leaf significantly. NAB and NNK were not reported in the combined analysis because of their low concentrations and relative insignificance to the total TSNA content in the cured leaf. TSNA content at individual locations and in the combined analysis show that as nitrogen fertilization increases, so does the concentration of TSNA in cured leaf

when all other production variables remain constant. This supports previous findings that nitrogen fertilization practices influence TSNA formation (MacKown et al., 1999; MacKown and Sutton, 1997; Burns, 1986; Marchetti et al., 2006; Atkinson and Sims, 1973; Aycock et al., 1979; Jones and Tramel, 1979; Broaddus et al., 1965; Hamilton et al., 1982; Elliot and Court, 1978; McKee, 1978; Link and Terrill, 1982; Bush et al., 2001; Wahlberg et al., 1999; Wiernik et al., 1995; Hamilton et al., 1982; Rundolf et al., 2000). However, it is thought that KT 204LC produces lower amounts of TSNA than other older varieties, and the TSNA analysis for 2008 will be able to compare TSNA among four varieties. Cured leaf samples for 2008 has not analyzed for TSNA analysis are currently at the University of Kentucky.

2007 Pre-sidedress Soil Nitrate Test (PSNT)

Previous research utilizing the PSNT has been conducted on corn, wheat and some other specialty crops to account for nitrogen that has mineralized at the beginning of the growing season in order to optimize nitrogen fertilization recommendations. In 2007, samples were taken from five sites: GR, SP, GL, LE and DS. The mean soil nitrate-N levels for preplant nitrogen rates are shown in Table 4.13. The PSNT was able to differentiate between the preplant nitrogen rates at all sites and this is supported by previous research on other crops (Magdoff, 1991; MacKown, 1999; Evanylo and Alley, Magdoff et al., 1990; Meisinger et al., 1992; Heckman et al., 2002; Kruseke et al., 2002; Waynick, 2007; Ma et al., 1999; Klausner et al., 1993). As the nitrogen fertilization rate increased soil nitrate concentrations increased. LE PSNT were not differentiated by mean separation; however, the preplant nitrogen effect was significant at that location ($P < 0.0364$). Since the PSNT can differentiate between the increasing preplant fertilization

Table 4.13: 2007 PSNT soil nitrate-N levels for individual locations

Preplant N	Sidedress N	GR	SP	GL	LE	DS
(kg/ha)	(kg/ha)	-----NO ₃ -N (ppm) -----				
0	-	36.8 c*	23.0 c	54.1 b	50.0	50.1 e
67	-	57.0 c	39.0 bc	83.9 ab	49.9	79.3 d
135	-	89.6 b	48.4 ab	94.0 ab	67.8	113.8 c
202	-	98.0 b	62.6 a	97.6 a	79.8	147.0 b
270	-	126.9 a	68.3 a	99.6 a	78.0	192.0 a

* Values with the same letters within the same locations are not significantly different at 0.1 level of probability

levels, and we know that yields are being maximized at 135 kg N/ha treatment, then a critical level should be able to be established.

2008 PSNT

In 2008, the highest preplant nitrogen rate was dropped from the study leaving the four preplant nitrogen rates found in Table 4.14. Table 4.14 shows the average PSNT values for each preplant nitrogen treatment in 2008 at all locations. Again at all locations preplant nitrogen treatments were differentiated by the PSNT. However, GR and LE have lower PSNT values than the previous year. At LE this is likely due to rotation, as the 2008 crop followed tobacco the previous year. At GR, it is apparently a difference in soils. GL also tends to be a little higher in soil nitrate concentrations than other sites within the study. This is most likely due to the rotation and crop management practices used at GL. At GL, the fields are not harvested for forage in non-tobacco years, only mowed. Also, tobacco from border row is not harvested, but rather mowed and left on the field. These practices could contribute to a buildup of soil nitrate from mineralization of added organic matter.

Table 4.14: 2008 PSNT soil nitrate-N levels for individual locations

Preplant N	Sidedress N	GR	SP	GL	LE
(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm)-----			
0	-	20.7 d*	27.1 c	63.5 d	14.4 c
67	-	31.0 c	35.1 b	85.1 c	19.1 c
135	-	41.3 b	41.4 b	103. b	24.8 b
202	-	52.4 a	51.4 a	136.6 a	36.7 a

* Values with the same letters are not significantly different within locations at 0.1 level of probability

Combined Analysis over years and locations for PSNT

Table 4.15 is the PSNT averages for preplant nitrogen treatments averaged over all locations for both years. As mentioned previously the PSNT was able to differentiate between preplant nitrogen treatments. Later in the chapter, PSNT values for individual sites and the combined dataset will be evaluated for the usefulness to predict burley tobacco yields.

2007 Petiole sap nitrate-N Analysis

Petiole nitrate concentrations have been used in specialty crops or crops from which plant sap can be extracted easily in order to make nitrogen fertilization recommendations based on plant nitrogen status. The concept is to take samples early enough in the growing season in order to evaluate the nitrogen status of the crop and still have time to correct a nitrogen deficiency without a loss of yield or value of the crop. In this study four petioles were used to extract enough plant sap to analyze using a Cardy® nitrate meter at pre-sidedress, topping and harvest. The pre-sidedress sampling date petiole sap nitrate-N will be analyzed to see if there is a

Table 4.15: Means for PSNT soil nitrate-N over all years and locations

Preplant N	Sidedress N	All Locations
(kg/ha)	(kg/ha)	NO ₃ -N (ppm)
0	-	38.7 d*
67	-	53.7 c
135	-	67.9 b
202	-	82.6 a

* Values with the same letters are not significantly different at 0.1 level of probability

correlation with yield while the other sampling dates occur too late in the growing season to use for nitrogen recommendations. However, the topping and harvest samples will be analyzed to see if there is a correlation with to TSNA content.

Pre-sidedress sampling interval

Out of the five sites in 2007, four of the five reported pre-sidedress petiole nitrate data. Samples were taken approximately three weeks after transplant and sidedress nitrogen was applied immediately after samples were taken. Petiole nitrate-N concentrations for individual locations can be found in Table 4.16. Only two of the locations show differences in petiole nitrate concentrations at this sampling interval. Preplant nitrogen rates at GR and SP did not differ in petiole nitrate-N concentrations. It is possible that the drought conditions at these two sites in 2007 interfered with the tobacco plant's ability to take up the nitrogen that was available in the soil profile. However, Caldwell (2007) did not see differences in petiole nitrate-N levels in burley tobacco at four weeks after transplanting. GL and DS both had significant differences in petiole nitrate-N between nitrogen fertilization treatments, $P = 0.001$ and $P = 0.0013$ respectively. At GL only the 0 kg N/ha treatment differed from the highest petiole nitrate-N

Table 4.16: Petiole sap NO₃-N concentration for pre-sidedress nitrogen rates at all locations in 2007

Preplant N (kg/ha)	Sidedress N (kg/ha)	GR	SP	GL	DS
----- NO ₃ -N (ppm) -----					
0	-	1353	1233	1072 b	739 c
67	-	1475	1184	1216 ab	773 bc
135	-	1413	1419	1231 a	826 ab
202	-	1329	1309	1319 a	829 ab
270	-	1371	1313	1319 a	874 a

* Values with the same letters within the same location are not significantly different at 0.1 level of probability

concentration. Petiole nitrate levels were lower at DS than at any other site in 2007 at this sampling interval. At both GL and DS petiole nitrate-N concentrations increase with increasing nitrogen fertilization and this supports previous research on other crops (Westerveld et al., 2003). At DS petiole nitrate-N concentrations did not respond to increasing nitrogen fertilization above 135 kg N/ha.

Pre-topping sampling interval

The next sampling interval was at just before topping in burley tobacco production. Topping is the removal of the apical bud, either by hand or mechanical methods, to force the plant to develop larger leaves so that yields are maximized. Again four petioles were taken from each plot, and two at a time were crushed to extract enough sap for analysis. Petiole sap nitrate-N concentrations at topping can be found in Table 4.17 for the individual nitrogen rates at all locations in 2007. Main effect means at topping for petiole nitrate-N can be found in Table 4.18. GR, SP and DS show differences in petiole nitrate-N concentrations across nitrogen treatments.

Table 4.17: Petiole sap NO₃-N concentration for pre-topping nitrogen rates at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS
(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
0	0	706 c	619 bc	771	260 b
0	67	931 ab	546 c	877	791 a
67	0	853 bc	736 abc	758	654 ab
67	67	909 ab	884 abc	862	935 a
135	0	924 ab	798 abc	1010	973 a
135	67	1008 ab	978 a	854	1038 a
202	0	971 ab	805 abc	970	1088 a
202	67	944 ab	969 ab	914	1124 a
270	0	956 ab	965 ab	1046	1104 a
270	67	1058 a	891 abc	1134	944 a

* Values with the same letters within the same location are not significantly different at 0.1 level of probability

Table 4.18: Petiole sap NO₃-N concentrations at pre-topping for the main treatment effects at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS
(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
0	-	819 c	583 b	824	526 c
67	-	881 bc	810 a	810	794 bc
135	-	966 ab	888 a	932	1005 ab
202	-	958 ab	887 a	942	1106 a
270	-	1007 a	928 a	1090	1024 ab
-	0	882 b	785	911	815 b
-	67	970 a	854	928	966 a

* Values with the same letters are not significantly different within a location at 0.1 level of probability

GL is the only location that did show any differences in petiole sap nitrate-N concentrations for individual, preplant, and sidedress nitrogen rates. At all three locations preplant treatments differed in petiole sap nitrate-N levels. At SP sidedress nitrogen treatments did not differ. GR and DS showed a response to sidedress nitrogen at 0 kg N/ha, and petiole sap nitrate-N concentrations showed no response to increasing nitrogen fertilization above 67 kg N/ha preplant + 67 kg N/ha sidedress treatment. This is reiterated by the preplant nitrogen means in Table 4.18, which show that at GR and DS petiole sap nitrate-N concentrations reach maximum levels at 135 kg N/ha. Petiole sap nitrate-N concentrations at DS and SP did not significantly increase after a total of 67 kg N/ha was applied. Petiole sap nitrate-N concentrations ranged from 546 parts per million (ppm) $\text{NO}_3\text{-N}$ to 1134 ppm $\text{NO}_3\text{-N}$ across all locations, except at the DS where the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment had an average of 260 ppm petiole sap $\text{NO}_3\text{-N}$. This shows that if nitrogen is not limiting the petiole sap nitrate-N concentrations in burley tobacco should be in this general range for that species.

Pre-Harvest sampling interval

DS did not report petiole sap nitrate-N data for the pre-harvest interval, so there are only three locations in Table 4.19 and Table 4.20. Table 4.19 gives the individual nitrogen treatment means for all locations and Table 4.20 lists the preplant and sidedress main effect means at all locations. At all locations preplant and sidedress treatments differed in petiole nitrate concentrations. At GR and SP petiole nitrate concentrations did not increase significantly with added nitrogen fertilization above the 67 kg N/ha preplant + 67 kg N/ha sidedress treatment. GL statistically maximized petiole nitrate concentrations at the 67 kg N/ha preplant + 0 kg N/ha sidedress. Looking at the main effects, when preplant nitrogen rates were averaged over both

Table 4.19: Petiole sap NO₃-N concentration for pre-harvest nitrogen rates at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS
(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
0	0	385 d	788 c	885 b	-
0	67	906 c	746 c	1233 ab	-
67	0	1044 bc	814 bc	1243 ab	-
67	67	1303 abc	1338 ab	1541 a	-
135	0	1115 abc	1209 abc	1588 a	-
135	67	1550 a	1363 a	1588 a	-
202	0	1338 abc	996 abc	1363 ab	-
202	67	1350 abc	1256 abc	1550 a	-
270	0	1425 ab	1245 abc	1713 a	-
270	67	1547 a	1406 a	1688 a	-

* Values with the same letters within the same location are not significantly different at 0.1 level of probability

Table 4.20: Petiole sap NO₃-N concentrations at pre-harvest for the main treatment effects at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS
(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
0	-	646 c	767 b	1059 c	-
67	-	1173 b	1076 ab	1392 b	-
135	-	1333 ab	1286 a	1456 ab	-
202	-	1344 ab	1126 a	1588 ab	-
270	-	1486 a	1326 a	1700 a	-
-	0	1061 b	1010 b	1358 b	-
-	67	1331 a	1222 a	1520 a	-

* Values with the same letters are not significantly different with a location at 0.1 level of probability

sidedress nitrogen rates, GR and GL did not have a significant increase in petiole sap nitrate-N levels above 135 kg N/ha. SP maximized petiole sap nitrate-N levels with 67 kg N/ha preplant when sidedress treatments were averaged. Petiole sap nitrate-N levels at harvest can not be used for nitrogen fertilization recommendations, but since nitrate has been correlated with TSNA content in cured burley tobacco these concentrations will be analyzed to see if there is any relationship with total TSNA content.

2008 Petiole sap nitrate-N Analysis

Pre-sidedress sampling interval

For the study in 2008 there were four locations: GR, SP, GL, and LE. DS was not in the study in 2008. TN 90LC was also added to the study to compare older burley variety to a more recent variety, KT 204LC. Means for the variety by preplant nitrogen rates are in Table 4.21 and the means for preplant nitrogen rates and the two varieties are in Table 4.22. The pre-sidedress sampling data showed the same inconsistencies as the 2007 data. GR and LE both showed that petiole sap nitrate-N levels differed between preplant nitrogen rate, $P = 0.0003$ and $P = 0.0054$ respectively. At GR and LE petiole sap nitrate-N concentrations did not increase significantly above the 67 kg N/ha preplant as nitrogen fertilization increased; this held true for both KT 204LC and TN90LC. SP and GL had no significant differences in petiole nitrate concentrations for the variety/nitrogen rate combinations, preplant nitrogen rate, or varieties at the pre-sidedress sampling interval. There was no difference in petiole nitrate concentrations between varieties at GR. LE has petiole nitrate concentrations that are four to five times higher than all other sites in 2008. These higher concentrations are found at every sampling interval. Since the petiole

Table 4.21: Pre-sidedress Petiole sap NO₃-N concentrations in KT 204LC and TN 90LC for all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
KT 204LC	0	-	1078 c*	1188	1269	4669 b
KT 204LC	67	-	1288 ab	1144	1225	5781 a
KT 204LC	135	-	1331 ab	1099	1338	5238 ab
KT 204LC	202	-	1394 a	1191	1300	5344 ab
TN 90LC	0	-	1149 bc	1129	1213	4563 b
TN 90LC	67	-	1294 ab	1314	1281	5356 ab
TN 90LC	135	-	1341 ab	1268	1281	5488 ab
TN 90LC	202	-	1388 a	1188	1288	5869 a

* Values with the same letters are not significantly different within the same location at 0.1 level of probability

Table 4.22: Pre-sidedress Petiole sap NO₃-N concentrations for main treatment effects at individual locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
-	0	-	1113 b	1158	1240	4616 b
-	67	-	1291 a	1229	1253	5569 a
-	135	-	1336 a	1183	1309	5363 a
-	202	-	1391 a	1189	1294	5606 a
KT 204LC	-	-	1273	1155	1283	5258
TN 90LC	-	-	1293	1224	1266	5319

* Values with the same letters are not significantly different within a location at 0.1 level of probability

nitrate concentrations are so much higher they will not be included in the combined analysis for each sampling interval.

Five Weeks After Transplanting (5WAT) sampling interval

In 2007, only one sampling interval was during a period in which the data would be practical to use in nitrogen fertilization recommendations. In order to expand the data set during the time period in which nitrogen fertilization could be adjusted or evaluated, in 2008 another sampling date was added at five weeks after transplanting (5WAT). At this time a producer could still add fertilizer that might have an impact on yield. Petiole nitrate concentration means with the mean separations at the 5WAT interval are reported in Table 4.23 for the variety by nitrogen rate treatment combinations. Three out of the four locations had differences in the means for all treatment combinations. GL is the only location that did not have differences in the variety by nitrogen rate treatment combinations. Petiole nitrate concentrations increased as nitrogen rates increased at all three locations that had differences. At GR the petiole nitrate concentrations ranged from 744 ppm $\text{NO}_3\text{-N}$ in TN 90LC with 0 kg N/ha preplant + 0 kg N/ha sidedressed to 984 ppm $\text{NO}_3\text{-N}$ in TN 90LC with 202 kg N/ha preplant + 67 kg N/ha sidedressed. At SP, petiole nitrate concentrations ranged from 864 ppm $\text{NO}_3\text{-N}$ in KT 204LC at 0 kg N/ha preplant + 0 kg N/ha sidedressed to 1079 ppm $\text{NO}_3\text{-N}$ with 202 kg N/ha preplant + 0 kg N/ha sidedressed. Petiole nitrate concentrations at GR showed no response to increasing nitrogen fertilization above the 67 kg N/ha preplant + 0 kg N/ha sidedress rate for both varieties in the study. Only the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment for KT 204LC had significantly lower petiole nitrate concentration than the highest nitrate concentrations observed at that location. At LE the 0 kg N/ha preplant + 0 kg N/ha sidedress treatment for TN 90LC was

Table 4.23: 2008 Petiole sap $\text{NO}_3\text{-N}$ concentration at 5WAT for all nitrogen rates at all locations

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- $\text{NO}_3\text{-N}$ (ppm) -----			
KT 204LC	0	0	765 cd	864 b	1000	4400 ab
KT 204LC	0	67	823 bcd	996 ab	950	5000 ab
KT 204LC	67	0	869 abcd	975 ab	961	5125 ab
KT 204LC	67	67	863 abcd	963 ab	899	5063 ab
KT 204LC	135	0	919 ab	983 ab	1006	5563 a
KT 204LC	135	67	901 abc	1015 ab	1026	4988 ab
KT 204LC	202	0	914 ab	1079 a	1049	5125 ab
KT 204LC	202	67	939 ab	1044 a	1075	5400 a
TN 90LC	0	0	744 d	926 ab	850	4088 b
TN 90LC	0	67	787 bcd	1006 ab	906	4450 ab
TN 90LC	67	0	849 abcd	983 ab	1011	4588 ab
TN 90LC	67	67	884 abcd	1043 a	998	5113 ab
TN 90LC	135	0	885 abcd	1026 ab	999	5038 ab
TN 90LC	135	67	913 abc	1019 ab	1031	5225 ab
TN 90LC	202	0	944 ab	964 ab	1014	4813 ab
TN 90LC	202	67	984 a	1020 ab	983	4825 ab

* Values with the same letters are not significantly different within a location at 0.1 level of probability

the only treatment that differed from the highest observed nitrate concentrations. Preplant nitrogen rate over all sidedress and varieties differed at all locations at the 5WAT sampling interval. Mean petiole sap nitrate-N concentrations for the main treatment effects can be found in Table 4.24. Sidedressing with 67 kg N/ha only showed a response in petiole sap nitrate-N at one location, SP. Only two weeks have passed since the sidedress treatments would have been applied at this time, so there might not have been enough time elapsed for the plants actively taking up the sidedress nitrogen at some locations. LE was the only site at this sampling time that had variety differences in petiole nitrate concentrations. At 5WAT, petiole nitrate concentrations at SP, GL, and LE were maximized at the 67 kg N/ha preplant rate. GR needed 135 kg N/ha in order to maximize petiole nitrate levels. Other than LE the maximum petiole nitrate concentrations were similar at the locations. Differences in petiole nitrate concentrations were able to be detected at this sampling date consistently, which may be useful in developing nitrogen fertilization recommendations based on petiole nitrate levels at this sampling date.

Pre-topping sampling interval

Petiole nitrate concentrations differed between individual treatments at all sites reported at the pre-topping sampling interval. Treatment means are reported in Table 4.25 along with the mean separation. LE did not report pre-topping petiole nitrate concentrations. At GR, there was a response to increasing nitrogen fertilization up to 135 kg N/ha with no response to added nitrogen above this rate. The petiole nitrate concentration at 67 kg/ha total nitrogen rate for both KT 204LC and TN 90LC at GR was statistically equal to the highest nitrate concentration found at this sampling date. Petiole sap nitrate-N concentrations at the 0 kg/ha total nitrogen declined from the 5WAT sampling interval to the pre-topping sampling interval at all sites. There was no

Table 4.24: Means for main treatment effects on petiole sap NO₃-N concentrations at 5WAT at all locations in 2008

Variety	Preplant N (kg/ha)	Sidedress N (kg/ha)	GR	SP	GL	LE
			----- NO ₃ -N (ppm) -----			
-	0	-	780 c*	948 b	927 b	4484 b
-	67	-	866 b	991 ab	967 ab	4972 a
-	135	-	905 ab	1011 a	1016 a	5203 a
-	202	-	945 a	1027 a	1030 a	5040 a
-	-	0	861	975 b	986	4842
-	-	67	887	1013 a	983	5008
KT 204LC	-	-	874	990	996	5083 a
TN 90LC	-	-	874	998	974	4767 b

* Values with the same letters are not significantly different within locations and main effects at 0.1 level of probability

Table 4.25: Petiole sap $\text{NO}_3\text{-N}$ concentrations at topping in all treatment combinations for all locations

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- $\text{NO}_3\text{-N}$ (ppm) -----			
KT 204LC	0	0	655 cd*	256 d	883 b	-
KT 204LC	0	67	873 abc	583 cd	1018 ab	-
KT 204LC	67	0	763 bcd	645 bc	1020 ab	-
KT 204LC	67	67	838 abcd	855 abc	1074 ab	-
KT 204LC	135	0	869 abc	825 abc	1015 ab	-
KT 204LC	135	67	996 ab	915 abc	1049 ab	-
KT 204LC	202	0	971 ab	700 abc	1000 ab	-
KT 204LC	202	67	994 ab	1006 a	1003 ab	-
TN 90LC	0	0	578 d	262 d	1021 ab	-
TN 90LC	0	67	958 ab	758 abc	1191 a	-
TN 90LC	67	0	806 bcd	646 bc	1008 ab	-
TN 90LC	67	67	988 ab	896 abc	1115 ab	-
TN 90LC	135	0	845 abcd	800 abc	1125 ab	-
TN 90LC	135	67	876 abc	918 abc	1138 ab	-
TN 90LC	202	0	993 ab	930 ab	1213 a	-
TN 90LC	202	67	1088 a	984 a	1138 ab	-

* Values with the same letters are not significantly different within a location at 0.1 level of probability

effect on petiole nitrate concentrations when split nitrogen applications were made compared to putting the total nitrogen rate down preplant at all locations. The predominant response in petiole nitrate was from increasing total nitrogen applied and not split or sidedress applications. Only the 0 kg N/ha preplant + 0 kg N/ha sidedress for KT 204LC differs from the highest petiole nitrate concentration at GL. Neither preplant or sidedress nitrogen treatments differed at GL, with $P = 0.5480$ and $P = 0.1034$ respectively. Means for the main treatment effects can be found in Table 4.26. GL was the only location at pre-topping to see a difference in petiole nitrate between varieties. At all locations TN 90LC was numerically higher in $\text{NO}_3\text{-N}$ than KT 204LC. Petiole nitrate concentrations did differ between preplant and sidedress rates at GR and SP. At both locations no response in petiole nitrate was observed above 135 kg N/ha preplant. The 67 kg N/ha sidedress treatments had higher petiole nitrate concentrations than the 0 kg N/ha rate over all preplant treatments. There was an interaction effect between preplant and sidedress treatments at GR and SP, $P = 0.0768$ for GR and $P = 0.0328$ for SP. This effect is seen because petiole nitrate levels increased with sidedress application at the two lowest preplant nitrogen treatments, but no significant increase in petiole nitrate levels is observed above the 135 kg N/ha preplant treatment. Since this sampling date is too late in the season to adjust fertilization for yield the differences observed in petiole nitrate will be evaluated as to their relationship to total TSNA content in the cured leaf.

Pre-Harvest sampling interval

All four locations in 2008 reported petiole sap nitrate-N data for pre-harvest sampling interval. Individual treatment means for petiole sap nitrate-N concentration at the harvest are shown in Table 4.27. Visual observations in the field at the time of sampling showed that the

Table 4.26: Means for the main treatment effects for Petiole NO₃-N concentrations at pre-topping for all location in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
-	0	-	766 c	465 c	1028	-
-	67	-	849 bc	761 b	1054	-
-	135	-	897 ab	864 ab	1082	-
-	202	-	1011 a	905 a	1088	-
-	-	0	810 b	633 b	1035	-
-	-	67	951 a	864 a	1090	-
KT 204LC	-	-	870	723	1008 b	-
TN 90LC	-	-	891	774	1118 a	-

* Values with the same letters are not significantly different within locations and main effects at 0.1 level of probability

Table 4.27: Petiole sap NO₃-N concentrations at pre-harvest for all treatment combinations for all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	----- NO ₃ -N (ppm) -----			
KT 204LC	0	0	388 e*	128 g	690 c	2463 d
KT 204LC	0	67	651 cde	328 efg	986 abc	3163 cd
KT 204LC	67	0	704 bcde	331 efg	1025 abc	3613 bcd
KT 204LC	67	67	869 abc	705 bcdef	1095 abc	5813 ab
KT 204LC	135	0	769 abc	514 defg	986 abc	4950 abc
KT 204LC	135	67	820 abc	944 abc	1199 ab	5163 abc
KT 204LC	202	0	934 abc	741 bcde	1109 abc	5388 abc
KT 204LC	202	67	979 ab	950 ab	1310 ab	6613 a
TN 90LC	0	0	448 de	120 g	860 bc	2363 d
TN 90LC	0	67	669 bcde	525 cdefg	1250 ab	3638 bcd
TN 90LC	67	0	669 bcde	299 fg	1074 abc	4163 bcd
TN 90LC	67	67	763 abcd	843 abcd	983 abc	5750 ab
TN 90LC	135	0	966 abc	606 bcdef	1103 abc	5363 abc
TN 90LC	135	67	1014 ab	903 abcd	1191 ab	5700 ab
TN 90LC	202	0	856 abc	848 abcd	1049 abc	6800 a
TN 90LC	202	67	1025 a	1200 a	1338 a	5688 ab

* Values with different letters are significantly different within a location at 0.1 level of probability

plots fertilized with 0 and 67 kg N/ha preplant were showing nitrogen deficiencies, chlorotic leaves and stunted growth. At SP, plants receiving 0 kg/ha total nitrogen could easily be picked out because of the chlorosis of the entire plant. Petiole nitrate levels at the three lowest nitrogen rates declined from the pre-topping sampling date (Table 4.25) petiole concentrations. The decline in petiole nitrate between the two sampling dates and visual appearance show that the plants did not have ample nitrogen available for optimum growth. GR, SP, and LE did not increase petiole nitrate concentrations above 135 kg/ha of total nitrogen for both varieties sampled. GL saw no response in petiole nitrate to added nitrogen above the 67 kg /ha of total nitrogen. GL has repeatedly maximized petiole nitrate levels at this treatment, which may be due to higher soil nitrate levels at this site compared to other sites (Table 4.14). Again split nitrogen applications had no effect on petiole nitrate concentrations when comparing total nitrogen applied. Having higher available nitrate in soil will diminish the response of tobacco to increasing nitrogen fertilization. Mean petiole nitrate concentrations for the main treatment effects can be found in Table 4.28. Preplant nitrogen main effect significantly differed in regards to petiole nitrate at harvest for all locations. GR and LE had a response to nitrogen fertilization up to 135 kg N/ha for the preplant. GL again, had no response to increasing nitrogen rates above the 67 kg N/ha preplant treatment. SP had the greatest response to nitrogen fertilization, petiole nitrate levels did not reach a maximum until the highest preplant nitrogen rate. Sidedress nitrogen treatment differed when averaged over all preplant treatments at every location. SP was the only site at pre-harvest to have a difference between varieties in petiole nitrate concentration. TN 90LC was higher in petiole nitrate than KT 204LC. Though not significantly different, TN 90LC petiole nitrate concentration were also higher than KT 204LC at GR, GL, and LE.

Table 4.28: Main treatment effect means petiole sap $\text{NO}_3\text{-N}$ concentrations at pre-harvest for all locations in 2008

Variety	Preplant N (kg/ha)	Sidedress N (kg/ha)	GR	SP	GL	LE
----- $\text{NO}_3\text{-N}$ (ppm) -----						
-	0	-	539 c*	275 d	945 b	2906 c
-	67	-	751 b	544 c	1044 ab	4834 b
-	135	-	892 a	742 b	1120 a	5294 ab
-	202	-	948 a	935 a	1201 a	6122 a
-	-	0	717 b	448 b	987 b	4388 b
-	-	67	849 a	800 a	1169 a	5191 a
KT 204LC	-	-	764	580 b	1050	4645
TN 90LC	-	-	801	668 a	1106	4933

* Values with the same letters are not significantly different within locations and main effects at 0.1 level of probability

Petiole sap nitrate-N at Pre-harvest for Three Varieties by Five Total Nitrogen Treatments

In addition to taking samples for KT 204LC and TN 90LC at all nitrogen rates in 2008 at the pre-harvest sampling interval, TN 90HC was also sampled for petiole sap nitrate-N concentration over the five total nitrogen rates it received. This analysis focuses on variety differences as well as the effect of total nitrogen on petiole nitrate levels. Table 4.29 has the individual treatment means for varieties by total nitrogen. LE is not reported in this table because of the elevated petiole nitrate levels reported in the previous petiole nitrate analysis. LE petiole nitrate data for this analysis was also not included in the combined analysis because the elevated nitrate concentrations would skew the means for GR, GL, and SP combined analysis reported in Table 4.30. Petiole nitrate concentrations in KT 204LC and TN 90LC at GR did not respond to nitrogen fertilization above 135 kg total N/ha treatment. TN 90 HC effectively maximized petiole nitrate concentrations at the 67 kg total N/ha rate at GR. There was no response to additional nitrogen above the 135 kg total N/ha rate at SP for all varieties. GL was the least responsive site to nitrogen fertilization, where petiole nitrate concentration showed no significant increase past 67 kg total N/ha. The total nitrogen main effect was significant at all sites. The means for the total nitrogen main effects we see SP and GR increased in petiole nitrate concentrations up to 202 kg total N/ha. Above 202 kg total N/ha there was no response to additional nitrogen at these sites. Petiole nitrate concentrations increased over all varieties up to 67 kg total N/ha at GL; above this rate no significant increases in petiole nitrate concentrations were observed. The only site where varieties differed in petiole nitrate at harvest was GL $P=0.100$. TN 90HC contained higher petiole nitrate levels than KT 204LC. Though the means were not significantly different at SP and GR, TN 90HC and TN 90LC contained higher petiole

Table 4.29: Petiole sap $\text{NO}_3\text{-N}$ at pre-harvest for three varieties receiving five total nitrogen rates

Location	Variety	Total Nitrogen Applied					Variety
		0	67	135	202	270	Avg. NO ₃ -N
----- (kg/ha) -----							(ppm)
GR	KT 204LC	388 e*	639 cde	869 abc	820 abc	979 ab	739
	TN 90LC	448 de	700 bcd	753 abcd	980 ab	1025 a	781
	TN 90HC	448 de	802 abc	852 abc	1023 a	1015 a	828
	Avg. NO ₃ -N (ppm)	428 D	714 C	825 BC	941 AB	1006 A	
SP	KT 204LC	128 e	319 de	705 bcd	944 abc	950 abc	609
	TN 90LC	114 e	525 cde	979 abc	903 abc	1200 a	694
	TN 90HC	140 e	378 de	726 bcd	969 abc	1060 ab	705
	Avg. NO ₃ -N (ppm)	127 D	407 C	803 B	938 AB	1070 A	
GL	KT 204LC	679 e	986 bcde	1095 abcd	1199 abcd	1304 abc	1053 B
	TN 90LC	860 de	1250 abcd	1363 ab	1191 abcd	1338 abc	1124 AB
	TN 90HC	684 e	1046 abcde	983 cde	1244 abcd	1400 a	1147 A
	Avg. NO ₃ -N (ppm)	741 C	1094 B	1147 AB	1211 AB	1347 A	

* Values with the same letters are not significantly different within locations and main effect means at 0.1 level of probability

Table 4.30: Petiole sap NO₃-N concentrations at pre-harvest for three varieties receiving five total nitrogen rates

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Location	Variety	Total Nitrogen Applied					Variety
		0	67	135	202	270	Avg. NO ₃ -N
		----- (kg/ha) -----					(ppm)
All Sites	KT 204LC	400 f*	649 de	890 bcd	988 abc	1079 ab	801 B
	TN 90LC	488 ef	825 cd	822 cd	1025 abc	1188 a	869 A
	TN 90HC	424 ef	741 d	1079 ab	1085 ab	1158 a	897 A
	Avg. NO ₃ -N (ppm)	437 D	738 C	930 B	1033 AB	1141 A	

* Values with the same letters are not significantly different within locations and main effect means at 0.1 level of probability

nitrate concentrations than KT 204LC over all nitrogen rates at pre-harvest.

The combined analysis results across the three locations are reported in Table 4.30. Petiole nitrate concentrations in KT 204LC and TN 90HC increased up to 135 kg total N/ha, but showed no response to additional nitrogen above this rate. TN 90LC increased in petiole nitrate up to 202 kg total N/ha, but did not significantly increase above this nitrogen rate. Total nitrogen treatments over all varieties differed in petiole nitrate concentration ($P < 0.0001$). Petiole nitrate increased with increasing nitrogen fertilization over all varieties up to 202 total kg N/ha. Varieties did differ ($P = 0.0037$) when petiole nitrate concentrations were averaged over all total nitrogen rates and locations. KT 204LC accumulated lower petiole nitrate than TN 90LC and TN 90HC. This is important because nitrate is a key component in the TSNA formation process. KT 204LC would have the potential to accumulate lower TSNA than TN 90LC or TN 90HC. TSNA samples were collected for all varieties in the study, and are currently at the University of Kentucky for analysis.

Combined Petiole sap nitrate-N Analysis

Petiole nitrate-N concentration data were combined over the two years and all locations and analyzed with only the eight nitrogen rates that were common in both years and for KT 204LC only. At the 5WAT sampling time only GR, GL, and SP in 2008 were used because this sampling time was not in the 2007 study. Also, LE data in 2008 were not included in the combined analysis at any sampling times due to their extremely high petiole nitrate-N concentrations.

Pre-sidedress sampling interval

Petiole nitrate-N concentrations differed among preplant nitrogen rates over all locations ($P = 0.0032$). Means for preplant nitrogen rate can be found in Table 4.3. Petiole nitrate-N concentration increased with increasing nitrogen application with means ranging from 1135 ppm $\text{NO}_3\text{-N}$ for the 0 kg N/ha preplant rate to 1239 ppm $\text{NO}_3\text{-N}$ for the 202 kg N/ha preplant treatment. The 0 kg N/ha treatment was significantly lower in petiole $\text{NO}_3\text{-N}$ than the 135 and 202 kg N/ha treatments at the pre-sidedress sampling interval. There was no increase in petiole nitrate-N concentrations above 67 kg N/ha preplant.

5WAT sampling interval

Compared to the pre-sidedress petiole nitrate-N concentrations for the preplant rates, the 5WAT petiole nitrate-N concentrations as a whole are lower. Individual and main effect treatment means can be found in Table 4.32 along with the mean separation. Petiole nitrate concentrations increase with increasing total nitrogen rates at the 5WAT interval. There is no difference in sidedress treatments averaged across all preplant rates ($P = 0.5288$). Preplant nitrogen rates averaged across both sidedress rates did differ in petiole nitrate concentrations ($P < 0.0001$). There was no increase in petiole $\text{NO}_3\text{-N}$ above 135 kg/ha total nitrogen. Petiole sap $\text{NO}_3\text{-N}$ for KT 204LC ranged from 876 ppm to 1019 ppm at 5WAT. Since differences are visible at 5WAT in petiole nitrate-N concentration there may be potential to be able to make nitrogen fertilization recommendations based on petiole nitrate at this point in the growing season for burley tobacco.

Table 4.31: Pre-sidedress petiole sap $\text{NO}_3\text{-N}$

concentrations for KT 204LC over all locations and years

Preplant N (kg/ha)	Sidedress N (kg/ha)	
	0	67
	----- $\text{NO}_3\text{-N}$ (ppm)-----	
0	1135 b*	-
67	1187 ab	-
135	1237 a	-
202	1239 a	-

* Values with the same letter are not significantly different at 0.1 level of probability

Table 4.32: Combined 5WAT petiole sap $\text{NO}_3\text{-N}$ concentration for KT 204LC over all locations

Preplant N (kg/ha)	Sidedress N (kg/ha)		Preplant Avg. $\text{NO}_3\text{-N}$
	0	67	
	----- $\text{NO}_3\text{-N}$ (ppm)-----		(ppm)
0	876 d*	923 cd	900 B
67	935 bcd	908 cd	921 B
135	969 abc	981 abc	975 A
202	1014 ab	1019 a	1016 A
Sidedress Avg.	948	958	

* Values with the same letter are not significantly different within locations and main effects at 0.1 level of probability

Pre-topping sampling interval

The treatments with 0 and 67 kg/ha total nitrogen were lower at this sampling period than their respective treatment at the 5WAT sampling period. Means for individual nitrogen treatments and the main treatment effects for the combined pre-topping sampling interval can be found in Table 4.33. Petiole sap $\text{NO}_3\text{-N}$ increased with increasing nitrogen up to 135 kg/ha total N, but there was no significant increase above this nitrogen rate. There was no difference in petiole sap nitrate-N levels at a given total nitrogen rate whether the nitrogen was applied in split applications or all nitrogen applied preplant. When averaged over both sidedress nitrogen rates KT 204LC differed in petiole sap $\text{NO}_3\text{-N}$ concentration. Over all preplant treatments sidedress treatments differed in petiole sap $\text{NO}_3\text{-N}$ levels ($P < 0.0001$). There was a preplant by sidedress interaction among individual means at pre-topping ($P = 0.0809$). This interaction primarily is caused by the large difference in the 0 and 67kg N/ha preplant with no sidedress and the 0 and 67kg N/ha preplant with sidedress. The sidedressed 0 and 67 kg N/ha preplant treatments are significantly different from the non-sidedress treatments, while there is no difference observed at the 135 and 202 kg N/ha treatments between sidedressed and non-sidedressed means.

Pre-harvest sampling interval

Means for individual treatments and main effects can be found in Table 4.34. The only treatment that continued to decrease over the course of the growing season was the 0 kg/ha total nitrogen. At the pre-harvest interval, the petiole nitrate-N concentrations increase up to 135 kg/ha total nitrogen applied and do not increase with added nitrogen above this rate. Petiole sap $\text{NO}_3\text{-N}$ increased in all nitrogen rates from topping to harvest except the 0 kg/ha total nitrogen.

Table 4.33: Pre-topping petiole sap $\text{NO}_3\text{-N}$ concentrations for

KT 204LC over all locations and years

Preplant N (kg/ha)	Sidedress N (kg/ha)		Preplant Avg. $\text{NO}_3\text{-N}$ (ppm)
	0	67	
	$\text{NO}_3\text{-N}$ (ppm)		
0	602 d	802 bc	702 C
67	780 c	907 ab	843 B
135	914 ab	975 a	945 A
202	924 a	989 a	956 A
Sidedress Avg.	805 B	918 A	

* Values with same letter are not significantly different at 0.1 level of probability

Table 4.34: Pre-harvest petiole sap $\text{NO}_3\text{-N}$ concentrations for

KT 204LC over all locations and years

Preplant N (kg/ha)	Sidedress N (kg/ha)		Preplant Avg. $\text{NO}_3\text{-N}$ (ppm)
	0	67	
	$\text{NO}_3\text{-N}$ (ppm)		
0	542 e	806 d	674 C
67	860 cd	1143 ab	1001 B
135	1030 bc	1244 a	1137 A
202	1080 ab	1232 a	1156 A
Sidedress Avg.	878 B	1106 A	

* Values with the same letter are not significantly different at 0.1 level of probability

Maximum nitrate-N levels at pre-harvest are in the same range as petiole sap nitrate-N levels at pre-sidedress. This makes sense because the apical dominance of the tobacco plant is removed at topping to force nutrients to the leaves in order to maximize biomass production. Sampling dates were not analyzed against other sampling dates, but this relationship might be of interest in the future. Petiole nitrate-N concentrations ranged from 542 ppm $\text{NO}_3\text{-N}$ for the 0 kg N/ha preplant with no sidedress to 1232 ppm $\text{NO}_3\text{-N}$ for the 202 kg N/ha with 67 kg N/ha sidedressed. Preplant nitrogen rates differed when averaged over both sidedress rates ($P < 0.0001$). Preplant nitrogen rate means ranged from 674 ppm $\text{NO}_3\text{-N}$ for the 0 kg N/ha rate to 1156 ppm $\text{NO}_3\text{-N}$ for the 202 kg N/ha rate. The main sidedress treatment effects differed in petiole nitrate-N concentrations with the 67 kg N/ha treatment higher than the 0 kg N/ha (Table 4.34).

Petiole nitrate-N levels differed among nitrogen treatments at every sampling date when data from all locations were combined. At the pre-sidedress sampling interval only the 0 kg N/ha preplant rate was different from the highest observed petiole nitrate. If there is a nutrient deficiency at this point in the growing season the petiole nitrate test could be used see if nitrogen may be what is deficient. At 5WAT differences in petiole nitrate were observed and this time period would be the best to evaluate nitrogen status of the crop and efficiently predict if more fertilizer needs to be applied. At this time in the growing season producers could apply more nitrogen if the crop petiole nitrate-N was lower than what is needed for optimum yields. When all locations were combined petiole nitrate did not significantly increase above 135 kg/ha total nitrogen applied at any of the sampling dates. From the results, 135 kg/ha total nitrogen, either applied all at preplant or in split applications, is the minimum amount of nitrogen burley tobacco needs to maintain the health of the crop.

2007 Leaf Chlorophyll Analysis

Leaf chlorophyll content has been used to evaluate nitrogen status in many crops such as corn and small grains. In corn and wheat the use of chlorophyll meters to monitor nitrogen status has gained favor in recent years it is a non-destructive way to measure chlorophyll content. Traditional lab techniques require tissue destruction and timely analysis procedures in the lab. Chlorophyll meters take an instantaneous reading in the field and do not destroy plant tissue. There are two types of meters in uses: reflectance and absorbance. Reflectance type meters measure light reflected and absorbance meters measure how much light passes through the plant. At GR, SP, GL, and LE a reflectance meter, Spectrum's CM 1000®, was used. At DS, both types were used, the GreenSeeker® by N Tech Industries which is a reflectance meter and the SPAD 502® meter manufactured by Minolta which is an absorbance meter.

Pre-sidedress sampling interval

In 2007 there were four locations where chlorophyll meter results are reported: GR, SP, GL, and DS. The means for the individual treatments at the pre-sidedress sampling interval are in Table 4.35. At GR, SP and GL the values are reported as chlorophyll index values which are unit-less measurements. At DS there are two values reported; NDVI is from the Greenseeker® and SPAD is from the the SPAD 502® meter. Both of these meters output measurements that are unit-less. GR, SP, and GL did not show any difference in chlorophyll index values at varying preplant rates of nitrogen at pre-sidedress. There was a difference in NDVI at DS between preplant nitrogen rates. The NDVI decreased as preplant nitrogen rates increased, which in conflict with research on other crops where NDVI increases with increased nitrogen fertilization (Blackmer and Schepers, 1994; Blackmer and Schepers, 1995; Schepers et al., 1992).

Table 4.35: Pre-sidedress leaf chlorophyll index at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS	
(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			NDVI	SPAD
0	-	184	152	184	0.689 a*	34.1
67	-	179	151	184	0.682 a	34.5
135	-	186	152	196	0.610 ab	34.5
202	-	186	151	189	0.617 ab	34.3
270	-	187	149	191	0.549 b	35.2

* Values with the same letter are not significantly different within location at 0.1 level of probability

SPAD readings at DS did not differ over the preplant nitrogen rates. At pre-sidedress NDVI was the only chlorophyll meter that showed significant differences among nitrogen rates.

Pre-topping sampling interval

Table 4.36 shows the means for chlorophyll measurements at all locations for the individual nitrogen treatments at pre-topping. GR, SP and GL had no differences in chlorophyll index readings over all individual nitrogen rates. At the pre-topping interval the chlorophyll index values at all three sites were higher than at the pre-sidedress interval. Means for the main treatment effect are in Table 4.37. Chlorophyll index values differed over the preplant main effects at pre-topping at GR ($P=0.0828$). Though chlorophyll index values differed there was no clear relationship between preplant nitrogen rate and chlorophyll index. SP and GL did not have any significant differences in the preplant main effects for chlorophyll index. Means for sidedress main treatment effects did not differ at GR and SP. Chlorophyll index means for the sidedress main treatment effect did differ at GL ($P=0.0363$). The 0 kg N/ha sidedress treatment had a higher chlorophyll index than the 67 kg N/ha sidedress treatment. This is the opposite of

Table 4.36: Pre-topping leaf chlorophyll index at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS	
(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			NDVI	SPAD
0	0	248	314	303	0.705	43.2 b*
0	67	260	324	281	0.682	46.4 ab
67	0	261	347	291	0.689	47.8 a
67	67	255	308	284	0.669	48.2 a
135	0	267	330	304	0.639	47.0 ab
135	67	275	350	290	0.570	48.7 a
202	0	260	325	324	0.642	47.9 a
202	67	252	357	300	0.612	49.6 a
270	0	256	332	341	0.573	49.7 a
270	67	250	343	290	0.591	49.1 a

* Values with the same letter are not significantly different within location at 0.1 level of probability

Table 4.37: Pre-topping sampling interval preplant and sidedress effects for leaf chlorophyll index at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS	
kg/ha	kg/ha	---- Chlorophyll Index ----			NDVI	SPAD
0	-	254 ab*	319	292	0.693 a	44.8b
67	-	258 ab	327	288	0.679 ab	48.0 a
135	-	271 a	340	297	0.604 bc	47.8 a
202	-	256 ab	341	312	0.627 abc	48.7 a
270	-	253 b	337	316	0.582 c	49.4 a
-	0	259	330	313 a	0.649	47.1 b
-	67	258	336	289 b	0.625	48.4 a

* Values with the same letter are not significantly different within location at 0.1 level of probability

the expected result because a plant that receives more nitrogen should be greener in color, therefore reflecting less red light and resulting in a higher reading. SPAD values increased with increasing nitrogen rates at DS and ranged from 43.2 at the 0 kg/ha total nitrogen to 49.7 at the 270 kg N/ha preplant + 0 kg N/ha sidedress. No significant differences were seen for NDVI at DS; however NDVI values decreased again with increasing nitrogen. Preplant and sidedress nitrogen main effects were significantly different in regards to SPAD values at DS. SPAD values increased up to 67 kg N/ha preplant and showed no response to added nitrogen above this rate. The sidedress treatments had higher SPAD values when averaged over all preplant rates. NDVI differed over preplant rates with the 0 kg N/ha having the highest NDVI and 270 kg N/ha having the lowest NDVI measurements ($P=0.0125$). NDVI did not differ over sidedress main effect rates.

Pre-harvest sampling interval

DS did not report pre-harvest data for NDVI and SPAD in 2007. At pre-harvest chlorophyll index did not differ over individual nitrogen rates at all locations. Means for individual nitrogen are in Table 4.38 and preplant and sidedress main treatment effect means are in Table 4.39. No differences were seen in preplant and sidedress main effects at any location. When burley tobacco is topped, it is also sprayed with growth regulators. These growth regulators are used to suppress growth of axillary buds in order to maximize leaf growth and expansion. One of the chemicals used to suppress axillary buds is maleic hydrazide, commonly referred to as MH. Usually applied with a high-boy sprayer; the chemical yellows the top leaves of the tobacco plant. Since we are measuring the upper most leaf at this sampling interval, we are measuring yellowed leaves that have been treated with MH. This results in unreliable

Table 4.38: Pre-harvest leaf chlorophyll index for all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS	
(kg/ha)	(kg/ha)	Chlorophyll Index			NDVI	SPAD
0	0	189	246	164	-	-
0	67	206	269	168	-	-
67	0	195	253	164	-	-
67	67	204	291	175	-	-
135	0	195	292	184	-	-
135	67	190	296	162	-	-
202	0	211	286	150	-	-
202	67	201	279	168	-	-
270	0	205	270	164	-	-
270	67	201	266	162	-	-

* Values with the same letter are not significantly different within location at 0.1 level of probability

Table 4.39: Pre-harvest preplant and sidedress effects on leaf chlorophyll index at all locations in 2007

Preplant N	Sidedress N	GR	SP	GL	DS	
(kg/ha)	(kg/ha)	Chlorophyll Index			NDVI	SPAD
0	-	198	258	166	-	-
67	-	200	272	170	-	-
135	-	193	294	173	-	-
202	-	206	282	159	-	-
270	-	203	268	163	-	-
-	0	199	269	165	-	-
-	67	200	280	167	-	-

* Values with the same letter are not significantly different within location at 0.1 level of probability

chlorophyll measurements since leaf chlorophyll content has been altered.

2008 Leaf Chlorophyll Analysis

In 2008, LE reported chlorophyll data, and used the same meter as GR, GL, and SP. DS did not participate in the study in 2008. A sampling interval was added at 5WAT and the harvest sampling interval was removed because of the use of MH. The 5WAT sampling interval was added to evaluate another time period during the growing season that could be used in nitrogen fertilization recommendations.

Pre-sidedress sampling interval

Chlorophyll index did not differ over individual nitrogen by variety treatments at all locations reported for the pre-sidedress sampling interval. GL was not reported because the chlorophyll meter did not record the data when measurements were being taken. Means for the individual nitrogen by variety for all locations are reported in Table 4.40. Main effect means for preplant and variety are in Table 4.41 along with mean separations for all locations. Chlorophyll index values differed with preplant nitrogen at GR ($P=0.0982$). Means for chlorophyll index at GR increased with increasing preplant nitrogen fertilizer and ranged from 173 at 0 kg N/ha to 179 at 202 kg N/ha (Table 4.41). The mean separation did not separate the means for preplant treatments in Table 4.41. There was no response at the other locations to preplant nitrogen at this sampling date. Chlorophyll index values did not differ among varieties at all locations at this sampling interval. Even when there were differences in chlorophyll index values the range between differing values would make it difficult to use confidently as a nitrogen management tool at this time during the season.

Table 4.40: Pre-sidedress leaf chlorophyll index for all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			
KT 204LC	0	-	173	164	-**	172
KT 204LC	67	-	179	168	-	173
KT 204LC	135	-	179	168	-	176
KT 204LC	202	-	179	164	-	174
TN 90LC	0	-	173	160	-	170
TN 90LC	67	-	169	164	-	171
TN 90LC	135	-	179	164	-	174
TN 90LC	202	-	178	164	-	167

** Meter did not record chlorophyll index values at this site

Table 4.41: Pre-sidedress sampling date preplant nitrogen and variety effects on leaf chlorophyll index at all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			
-	0	-	173	162	-**	171
-	67	-	174	166	-	172
-	135	-	179	166	-	175
-	202	-	179	164	-	170
KT 204LC	-	-	177	166 a*	-	174
TN 90LC	-	-	175	163 b	-	171

* Values with the same letter are not significantly different within location at 0.1 level of probability

** Meter did not record chlorophyll index values at this site

5WAT sampling interval

GR, SP, GL, and LE reported leaf chlorophyll measurements at this sampling interval in 2008. Of those four locations GR was the only one with chlorophyll index values that differed among individual variety by nitrogen rate treatments. Table 4.42 lists the means for variety by nitrogen rate treatments with the respective mean separations. Chlorophyll index values at GR increased with increasing nitrogen rates for both KT 204LC and TN 90LC. Only the two 0 kg N/ha preplant rates for KT 204LC significantly differed from the 202 kg N/ha preplant + 67 kg N/ha sidedress rate for TN 90LC. Chlorophyll index values at GR ranged from 165 to 185. SP and GL chlorophyll index values are substantially higher than the values reported at GR and LE. Table 4.43 lists the main treatment effect means and mean separations for variety, preplant, and sidedress nitrogen treatments. Chlorophyll index values differed among preplant nitrogen rates when averaged across the two sidedress nitrogen rates ($P = 0.0167$) at GR only. Chlorophyll index values showed no response to increasing preplant nitrogen above 67 kg N/ha (Table 4.41). Varieties at GR also differed in chlorophyll index values ($P = 0.005$). TN 90LC had higher chlorophyll index values than KT 204LC across all nitrogen treatments. No other location had significant effects on chlorophyll index values for varieties, preplant nitrogen, or sidedress nitrogen treatments.

Pre-topping sampling interval

Only GR, SP and GL reported chlorophyll data for the pre-topping sampling interval. Over all the locations chlorophyll index values increased from the 5WAT sampling interval to the pre-topping interval. This is an indication that the plants are actively growing and utilizing the nitrate available in the soil profile. Chlorophyll index means and mean separation for the

Table 4.42: 5WAT Leaf Chlorophyll index for all nitrogen by variety treatment in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			
KT 204LC	0	0	165 b*	240	205	161
KT 204LC	0	67	165 b	238	209	158
KT 204LC	67	0	183 ab	246	210	158
KT 204LC	67	67	168 ab	238	219	158
KT 204LC	135	0	169 ab	249	211	161
KT 204LC	135	67	173 ab	247	215	161
KT 204LC	202	0	176 ab	247	213	162
KT 204LC	202	67	178 ab	241	201	159
TN 90LC	0	0	167 ab	234	204	161
TN 90LC	0	67	174 ab	219	212	157
TN 90LC	67	0	181 ab	242	206	156
TN 90LC	67	67	179 ab	239	205	163
TN 90LC	135	0	181 ab	237	209	154
TN 90LC	135	67	174 ab	243	202	164
TN 90LC	202	0	177 ab	235	210	163
TN 90LC	202	67	185 a	242	212	152

* Values with the same letter are not significantly different within location at 0.1 level of probability

Table 4.43: 5WAT variety, preplant nitrogen rate, and sidedress nitrogen rate effects on leaf chlorophyll index for all locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			
-	0	-	168 b*	233	208	159
-	67	-	178 a	241	210	159
-	135	-	174 ab	244	209	160
-	202	-	179 a	241	209	159
-	-	0	175	241	208	160
-	-	67	174	238	209	159
KT 204LC	-	-	172 b	243	210	160
TN 90LC	-	-	177 a	237	207	160

* Values with the same letter are not significantly different within location at 0.1 level of probability

pre-topping interval for variety by nitrogen rate treatments are in Table 4.44. GR is the only site where mean separation is reported for individual treatment combinations. However, there is no difference in chlorophyll index in the nitrogen rates for KT 204LC. The 0 kg N/ha preplant + 67 kg N/ha sidedress with TN 90LC has a chlorophyll index value of 372 and is significantly higher than the 202 kg N/ha preplant + 0 kg N/ha sidedress by TN 90LC treatment with a chlorophyll index value of 308. There is no clear pattern to the response in chlorophyll index values and there is probably not a true difference due to nitrogen treatments. Table 4.45 lists the main treatment effect means for individual sites at pre-topping. SP is the only site where preplant nitrogen treatments differed when averaged over both varieties and sidedress treatments ($P=0.0566$). As preplant nitrogen increased at SP chlorophyll index values also increased up to 67 kg N/ha. Sidedress nitrogen treatments at GR significantly differed in chlorophyll index values with 67 kg N/ha having higher chlorophyll index values than the 0 kg N/ha rate (Table 4.45). None of the sites show a significant difference in chlorophyll index values between KT 204LC and TN 90LC at pre-topping.

Combined Leaf Chlorophyll Analysis for all locations

A combined analysis of variance was conducted using data from all sites with chlorophyll index values reported for each sampling interval. Only the eight individual nitrogen rates common to both years and the data from KT 204LC were used in the analysis. At pre-sidedress, differences in chlorophyll index values were seen between preplant nitrogen rates. The 0 kg N/ha preplant rate had the lowest chlorophyll index of the preplant treatments and only differed from the 135 kg N/ha rate (Table 4.46). The difference between the highest chlorophyll index value and the lowest value is six index units. This makes using the CM 1000 chlorophyll meter

Table 4.44: Pre-topping leaf chlorophyll index for all treatments and locations in 2008

Variety	Preplant N	Sidedress N	GR	SP	GL	LE
	(kg/ha)	(kg/ha)	---- Chlorophyll Index ----			
KT 204LC	0	0	315 ab*	353	280	---
KT 204LC	0	67	351 ab	378	287	-
KT 204LC	67	0	322 ab	386	278	-
KT 204LC	67	67	339 ab	376	302	-
KT 204LC	135	0	328 ab	366	271	-
KT 204LC	135	67	340 ab	385	273	-
KT 204LC	202	0	337 ab	399	300	-
KT 204LC	202	67	349 ab	386	267	-
TN 90LC	0	0	319 ab	365	283	-
TN 90LC	0	67	372 a	358	322	-
TN 90LC	67	0	338 ab	373	273	-
TN 90LC	67	67	350 ab	386	278	-
TN 90LC	135	0	326 ab	381	283	-
TN 90LC	135	67	323 ab	383	288	-
TN 90LC	202	0	308 b	366	290	-
TN 90LC	202	67	350 ab	383	309	-

* Values with different letters are significantly different at 0.1 level of probability

** Data is not available at this location for the sampling date

Table 4.45: Pre-topping variety, preplant nitrogen rate, and sidedress nitrogen rate effects on leaf chlorophyll index for all locations in 2008

Variety	Preplant N (kg/ha)	Sidedress N (kg/ha)	GR	SP	GL	LE
Chlorophyll Index						
-	0	-	339	364 b*	293	-**
-	67	-	337	380 ab	282	-
-	135	-	329	379 ab	279	-
-	202	-	336	383 a	291	-
-	-	0	324 b	374	282	-
-	-	67	347 a	379	291	-
KT 204LC	-	-	335	379	282	-
TN 90LC	-	-	336	374	290	-

* Values with different letters are significantly different within locations and main effects at 0.1 level of probability

** Data is not available at this location for the sampling date

Table 4.46: Pre-sidedress leaf chlorophyll index values

for KT 204LC by nitrogen treatments at all locations combined

Preplant N (kg/ha)	Sidedress N (kg/ha)	
	0	67
----Chlorophyll Index----		
0	170 b*	-
67	172 ab	-
135	176 a	-
202	174 ab	-

* Values with different letters are significantly different at 0.1 level of probability

as a nitrogen management tool in burley tobacco questionable. Given the variability in the field, veins running through the leaf being measured, and other environmental factors chlorophyll measurements may vary greater than six units. Producers or consultants would have to take a high number of samples to ensure accurate recommendations. The differences seen in the data at pre-sidedress are most likely due to an extremely high number of measurements at all locations.

At the 5WAT sampling interval no differences were seen in chlorophyll index values between individual nitrogen treatments over all locations. Individual and main effect means for chlorophyll index values at 5WAT can be found in Table 4.47. The 0 kg N/ha preplant rate with no sidedress and with sidedress had lower chlorophyll index values than the other preplant by sidedress combinations though these differences are not significant. With no main treatment effects or individual nitrogen rates differences found in chlorophyll index, there is little possibility of using the CM 1000 chlorophyll meter as a nitrogen management at 5WAT.

At the pre-topping sampling there were differences in chlorophyll index values between individual nitrogen and main effect preplant nitrogen rates. The mean chlorophyll index values for individual and main treatment effects can be found in Table 4.48. The 0 kg N/ha preplant + 0 kg N/ha sidedress treatment had the lowest chlorophyll index value and this was significantly different from the 202 kg N/ha preplant + 0 kg N/ha sidedress treatment which had the highest chlorophyll index value. The difference between the lowest chlorophyll index value and the highest was 17 index units. This is greater than the difference observed at pre-sidedress, but still may be too narrow to accurately predict nitrogen status of the plant. Also, yield response to applying nitrogen at this point in the growing season will be minimal because there is not enough time between topping and harvest to allow for the plant to recover from a nutrient deficiency and increase biomass. In tobacco having too much nitrogen available to the plant at harvest can result

*Table 4.47:5WAT leaf chlorophyll index values for
KT 204LC by nitrogen treatments at all locations combined*

Preplant N (kg/ha)	Sidedress N (kg/ha)		Avg. C.I.
	0	67	
Chlorophyll Index			
0	193	192	193
67	199	196	197
135	198	199	198
202	199	195	197
Avg. C.I.	197	195	

*Table 4.48:Pre-topping leaf chlorophyll index values
for KT 204LC by nitrogen treatments at all locations combined*

Preplant N (kg/ha)	Sidedress N (kg/ha)		Avg. C.I.
	0	67	
Chlorophyll Index			
0	284 b*	292 ab	288 B
67	292 ab	289 ab	290 AB
135	290 ab	297 ab	293 AB
202	301 a	296 ab	298 A
Avg. C.I.	292	293	

* Values with the same letter are not significantly different at 0.1 level of probability

in adverse effects during curing. At the pre-harvest sampling interval, data is only from 2007 as the sampling interval was dropped from the study in 2008. Means for the pre-harvest chlorophyll index values at the individual nitrogen and main effect nitrogen rates are found in Table 4.49. No differences in chlorophyll index values were observed for individual nitrogen rates and the main treatment effects. This was due to the use of MH, a growth regulator, at topping in order to control axillary bud growth. As a result the upper most leaves are yellowed resulting in chlorophyll index values that can not be used. The results from all sampling dates and locations for leaf chlorophyll evaluation as a nitrogen management tool for burley tobacco are inconsistent from location to location and from one sampling interval to another.

Chlorophyll measurements have been proven in corn and small grains as useful tools to manage nitrogen fertilization, but our data suggests that the CM 1000 chlorophyll meter would not be accurate and consistent enough to manage nitrogen in burley tobacco using the same sampling methods that were used in this study. Further evaluations of this meter using different techniques may prove to be promising.

Grade Index for 2007 and 2008

Grade index data were calculated from the federal grades at SP and GR in 2007 and 2008. These were the only locations which report grade index in the study. In 2007, there were no differences in grade index over individual nitrogen rates at either location; means can be found in the appendix (Table A.1). In 2008, grade index did not differ over the variety by total nitrogen treatments (Table A.2) at GR. At SP grade index differed between varieties, with NC 7 having significantly higher grade index than the other three varieties (Table A.2). Individual

Table 4.49:Pre-harvest leaf chlorophyll index values for KT 204LC by nitrogen treatments at all locations combined

Preplant N (kg/ha)	Sidedress N (kg/ha)		Avg. C.I.
	0	67	
Chlorophyll Index			
0	200	215	207
67	204	223	214
135	224	216	219
202	215	216	216
Avg. C.I.	211	218	

* Values with the same letter are not significantly different at 0.1 level of probability

variety by nitrogen rates differed with regards to grade index, but not in a consistent pattern.

Total nitrogen rate did not have an effect on grade index at SP in 2008. Overall nitrogen had no effect on grade index in the years of this study, however previous studies have shown that nitrogen fertilization can improve the quality of burley tobacco.

Regression Analysis of Pre-Sidedress Interval measurements and Yield

The regression analysis for individual sites at the pre-sidedress sampling intervals used absolute yield as the y-variable. The combined analysis for all sites uses relative yield (RY) for the y-variable. Relative yield was calculated by taking the highest average yield at each site for the preplant nitrogen rates with no sidedress nitrogen applied and dividing all yields for preplant nitrogen rates with no sidedress nitrogen by the highest average yield mentioned above. It was important to use only the non-sidedressed plots because no sidedress nitrogen treatment was

applied at the time of sampling and yields for sidedressed plots do not accurately correspond to values at the pre-sidedress sampling interval.

PSNT vs Yield

Models, equations, p-values and R-squares for the regression models are given in Table 4.50 for 2007 and Table 4.51 for 2008. In 2007 there were only three sites that had significant regression models. At GR, both the linear and quadratic models were significant, and each model had R^2 of 0.383. LE and DS were the other locations where both the linear and quadratic models were significant. The quadratic model at LE had the highest R^2 value for all models in 2007, 0.398. Figure 4.1 shows this relationship between PSNT and yield at LE. Models for SP and GL were not significant. The drought in 2007 diminished the relationship between PSNT and yield at all locations; this is seen in the overall higher R^2 values for in 2008 where rainfall events were more frequent.

In 2008, all quadratic models were significant and all but one linear model was significant, the exception being SP for TN 90LC. At GR the quadratic coefficients for KT 204LC and TN 90LC were not significant, so the best model to use is the linear model. The KT 204LC linear model at GR had the highest R^2 value of any location and model. Figure 4.2 is the graph for the linear relationship between PSNT and yield at GR in 2008. The R^2 values for locations show moderate relationships for PSNT and yield at the various locations. It is apparent that as PSNT levels increase, so do yield levels. The quadratic models show a higher correlation between PSNT and yield in 2008. From knowledge of nitrogen and how it affects yield we know that at some point crops stop responding to added nitrogen and the analyses of yield for this study have shown that above 135 kg/ha total nitrogen no response in yield is seen. Therefore a

Table 4.50: Regression equations and parameters at each location in 2007 for PSNT vs Yield

Year	Location	Model	Equation	Model (Pr > F)	R ²
2007	GR	Linear	$y = 5.708x + 3369$	0.0066	0.343
		Quadratic	$y = 0.00429x^2 + 5.053x + 3390$ **	0.028	0.343
	SP	Linear	$y = 5.88x + 1755$	0.182	0.096
		Quadratic	$y = 0.167x^2 - 10.12x + 2074$	0.342	0.118
	GL	Linear	$y = 3.00x + 3546$	0.254	0.071
		Quadratic	$y = -0.11x^2 + 25.17x + 2596$	0.145	0.203
	LE	Linear	$y = 6.128x + 3122$	0.014	0.289
		Quadratic	$y = -0.123x^2 + 25.3x + 2469$	0.013	0.398
	DS	Linear	$y = 3.10x + 2482$	0.047	0.202
		Quadratic	$y = 0.0019x^2 + 2.63x + 2508$ **	0.146	0.202

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

Table 4.51: Regression equations and parameters at each location in 2008 for PSNT vs Yield

Year	Location	Variety	Model	Equation	Model (Pr > F)	R ²
2008	GR	KT 204LC	Linear	$y = 20.37x + 1695$	0.0001	0.682
		KT 204LC	Quadratic	$y = -.099x^2 + 27.59x + 1583$ **	0.0006	0.684
		TN 90LC	Linear	$y = 12.5x + 1848$	0.027	0.323
		TN 90LC	Quadratic	$y = -0.17x^2 - 24.5x + 1668$ **	0.091	0.329
	SP	KT 204LC	Linear	$y = 18.56x + 1856$	0.0115	0.376
		KT 204LC	Quadratic	$y = -0.975x^2 + 97.71x + 388.9$	0.0083	0.521
		TN 90LC	Linear	$y = 6.74x + 2183$	0.8155	0.131
		TN 90LC	Quadratic	$y = -0.46x^2 + 53.34x + 1174$	0.0299	0.443
	LE	KT 204LC	Linear	$y = 24.6x + 2100$	0.0075	0.411
		KT 204LC	Quadratic	$y = -.096x^2 + 79.04x + 1451$	0.0081	0.523
		TN 90LC	Linear	$y = 28.3x + 1724$	0.0006	0.581
		TN 90LC	Quadratic	$y = 0.262x^2 + 12.27x + 1923$	0.0032	0.588

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

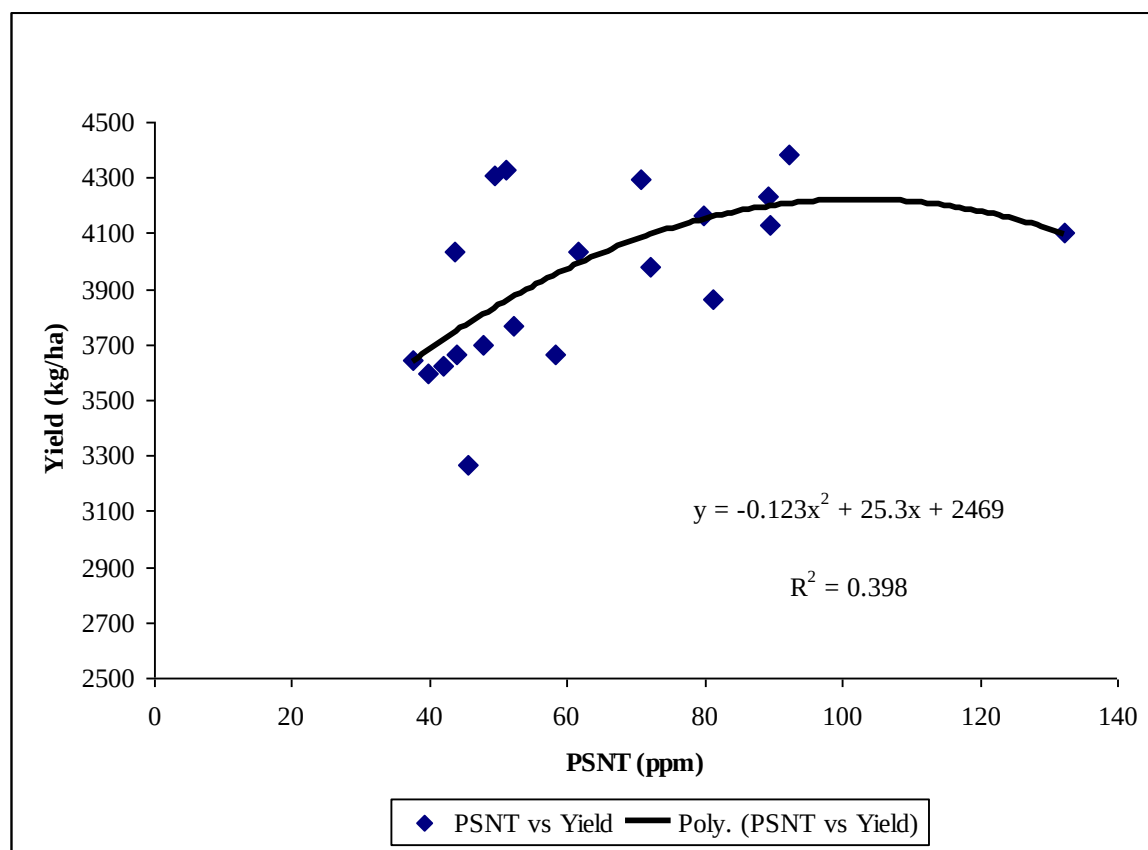


Figure 4.1: PSNT vs Yield at LE in 2007

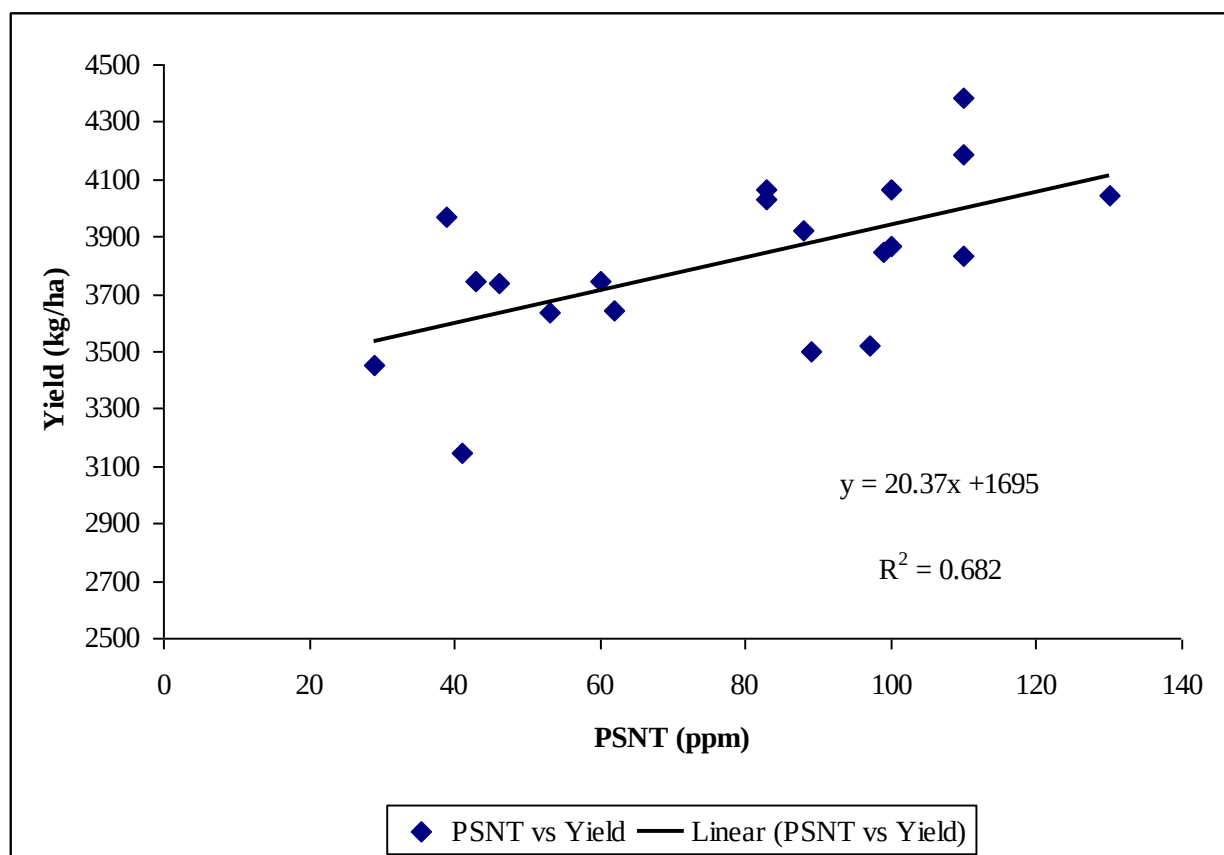


Figure 4.2: PSNT vs Yield at GR in 2008

quadratic plateau model would be a better model to evaluate PSNT versus yield.

Combined PSNT vs RY for all location over both years

All locations were combined and analyzed using the NLIN procedure in SAS 9.2. Instead of using absolute yields, yields were converted to relative yields for each location to normalize yields. One of the objectives of the study was to find a critical level, if possible, for PSNT where there would be no response in yield above a certain soil nitrate-N concentration. Figure 4.3 shows the combined PSNT regression equation explaining relative yield across all sites for both years. There is a clear plateau at a relative yield value of 0.946 and a PSNT value of 46.4 ppm nitrate-N. Table 4.15 has the combined PSNT values over all years and sites and this level of soil nitrate is reached at the 67 kg N/ha preplant. Waynick (2007) found that sites uniformly responded to added nitrogen when under 40 ppm soil nitrate-N, and sometimes responded up to 80 ppm soil nitrate-N. Burley tobacco would be expected to continue to respond past levels where other crops stop responding. The critical PSNT value for corn ranges from 20-30 ppm $\text{NO}_3\text{-N}$ (Magdoff, 1991; Magdoff et al., 1990; Binford et al., 1992; Heckman et al., 2002; Krusekopt et al., 2002; Rozas et al., 2000). However, these results were based on samples taken to a depth of 30 cm. The 15 cm depth samples used in this study would be expected to have a higher critical nitrate-N level. MacKown (1999) suggested two critical PSNT of 48 ppm and 88 ppm for two different soil types in Kentucky. These results correspond with previous research on burley tobacco and are averaged over multiple soil types. One might want to go back and separate data by soil types and to establish critical values for the predominant soil types used for burley tobacco production in Tennessee.

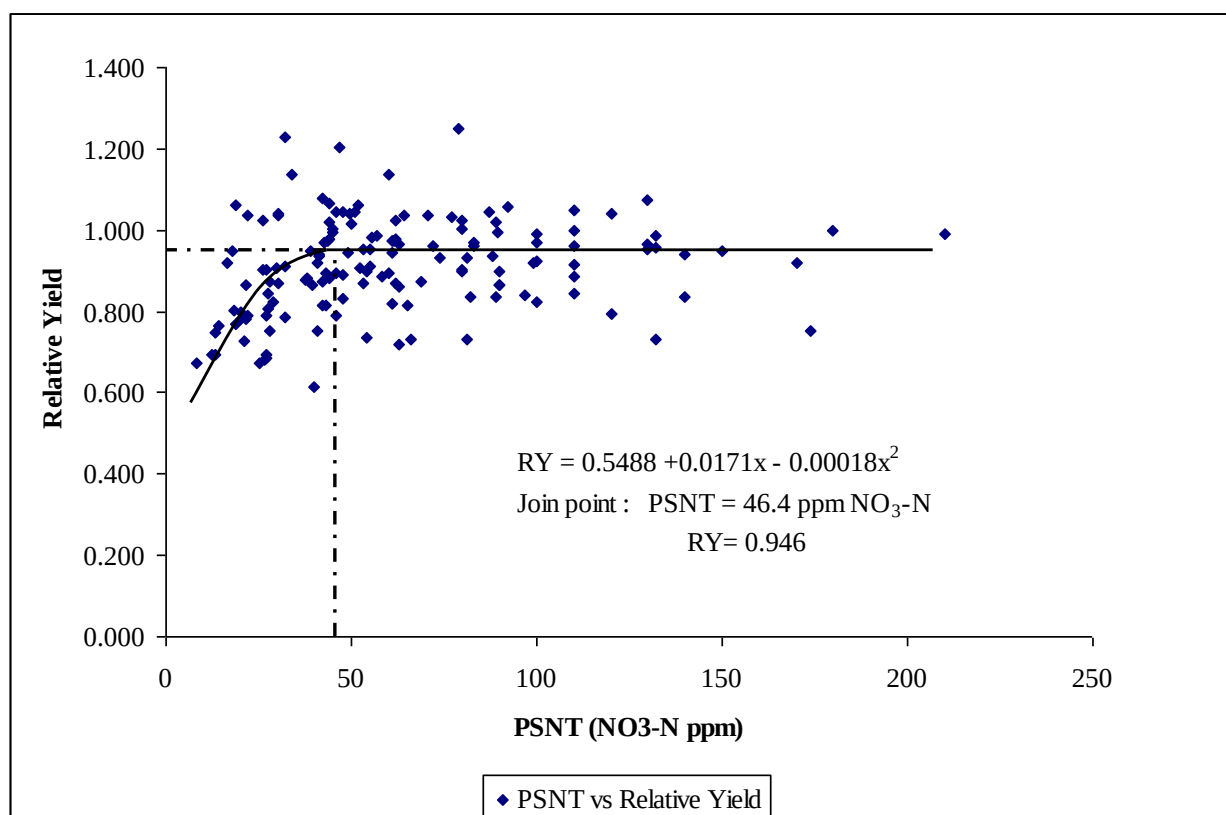


Figure 4.3: Quadratic Plateau Model for PSNT vs RY over all locations in 2007 and 2008

Regression Analysis for Petiole sap Nitrate-N versus Yield at Pre-sidedress

Petiole nitrate-N samples were plotted against absolute yield at individual locations to determine their correlation with yield. Table 4.52 has the equations and parameters for linear and quadratic models for petiole nitrate-N concentrations explaining yields in 2007. Table 4.53 has the same information for the locations in 2008. Only three equations were significant in 2007. At GR the quadratic model was significant and had a R^2 value of 0.448, which was the highest for any model for petiole nitrate explaining yield for any location over both years. The linear model at SP in 2007 was also significant and had a R^2 value of 0.388. GL had the last significant model in 2007 ($R^2=0.151$). In 2008, there were no models at any locations that were significant. Figure 4.4 is the scatter-plot for all locations combined for petiole nitrate concentration versus relative yield. The equations for the quadratic and linear models for the combined petiole nitrate regressions are at the bottom of Table 4.53. Both equations for the combined sites are significant, but the R^2 values indicate a weak relationship between petiole nitrate levels at pre-sidedress and yield levels. Given the consistent results of low R^2 and looking at the Figure 4.4, petiole nitrate levels at pre-sidedress would not be a good predictor of yield. The pre-sidedress sampling interval may be too early in the growing season to accurately determine whether the crop has enough nitrogen for optimum yield.

Regression Analysis for Chlorophyll Index versus Yield at Pre-sidedress

Chlorophyll index values were not consistently related to nitrogen rates across sampling intervals and locations. The regression analysis revealed this same trend when explaining yield by pre-sidedress chlorophyll index measurements. Tables 4.54 and 4.55 contain the equations, model p-values, and R^2 values for the linear and quadratic equations for chlorophyll index values

Table 4.52: Regression equations and parameters for petiole sap nitrate-N versus yields at pre-sidedress at all locations in 2007

Year	Location	Model	Equation	Model (Pr > F)	R ²
2007	GR	Linear	$y = -0.397x + 4354$	0.202	0.088
		Quadratic	$y = -0.0022x^2 + 5.968x - 196.90$	0.006	0.448
	SP	Linear	$y = 0.706x + 1132$	0.007	0.388
		Quadratic	$y = -0.00016x^2 + 1.072x + 942$	0.029	0.34
	GL	Linear	$y = 0.80x + 2809$	0.090	0.151
		Quadratic	$y = -0.00025x^2 + 1.394x + 2465$	0.247	0.152
	DS	Linear	$y = 0.408x + 2499$	0.683	0.0095
		Quadratic	$y = -0.0131x^2 + 21.45x - 8545$	0.528	0.0723

Table 4.53: Regression equations and parameters for petiole sap nitrate-N versus yields at pre-sidedress at all locations in 2008

Year	Location	Variety	Model	Equation	Model Pr > F	R ²
2008	GR	KT 204LC	Linear	$y = -0.045x + 2493$	0.9456	0.000
		KT 204LC	Quadratic	$y = 0.0039x^2 - 9.89x + 8602$	0.6854	0.056
		TN 90LC	Linear	$y = 0.192x + 2034$	0.6931	0.012
		TN 90LC	Quadratic	$y = -0.0018x^2 + 4.73x - 685$	0.7627	0.044
	SP	KT 204LC	Linear	$y = 0.82x + 1673$	0.1394	0.149
		KT 204LC	Quadratic	$y = 0.0021x^2 - 4.129x + 4490$	0.3136	0.163
		TN 90LC	Linear	$y = 0.25x + 2146$	0.5128	0.034
		TN 90LC	Quadratic	$y = -0.0025x^2 + 6.89x - 2123$	0.1207	0.297
	LE	KT 204LC	Linear	$y = 0.044x + 2436$	0.7293	0.009
		KT 204LC	Quadratic	$y = -0.0002x^2 + 2.76x - 4950$	0.3019	0.168
		TN 90LC	Linear	$y = 0.0067x^2 + 2403$	0.9421	0.000
		TN 90LC	Quadratic	$y = -0.000066x^2 + 0.785x + 225$	0.5436	0.090
All Sites***		KT 204LC	Linear	$y = 0.000077x + 0.845$	0.0632	0.031
		KT 204LC	Quadratic	$y = -0.00000012x^2 + 0.00035x + 0.692$	0.1063	0.040

***Combined analysis used relative yield at the y-variable

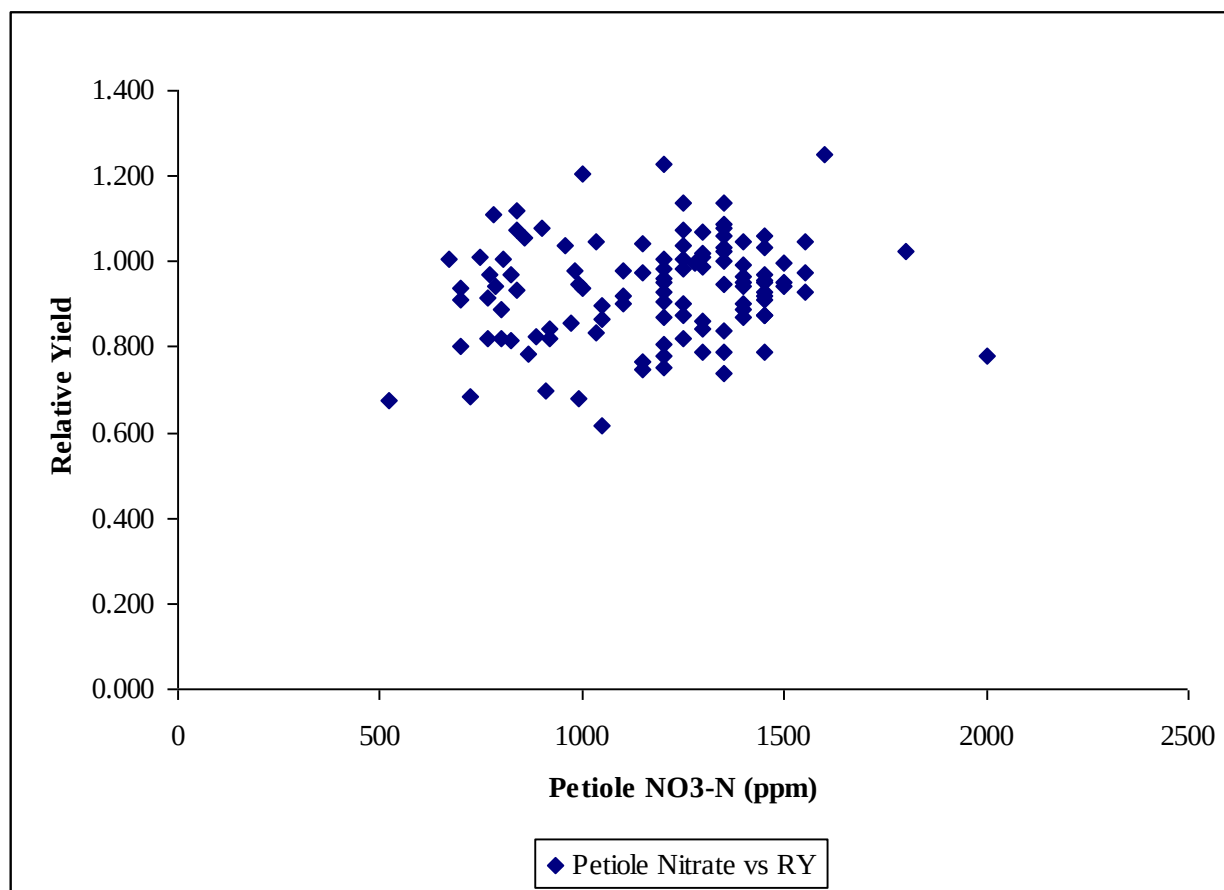


Figure 4.4: Petiole nitrate-N versus relative yield for KT 204LC at pre-sidedress for all locations

Table 4.54: Regression equations and parameters for chlorophyll index versus yield in 2007

Year	Location	Model	Equation	Model (Pr > F)	R ²
2007	GR	Linear	$y = 17.82x + 521.62$	0.0425	0.209
		Quadratic	$y = 0.377x^2 - 123.82x + 13820$	0.228	0.111
	SP	Linear	$y = 21.07x - 1092$	0.0052	0.19
		Quadratic	$y = 2.77x^2 - 778.48x + 56342$	0.0012	0.546
	GL	Linear	$y = 11.5x + 1637$	0.1335	0.121
		Quadratic	$y = 0.414x^2 - 140.69x + 15542$	0.227	0.159

Table 4.55: Regression equations and parameters for chlorophyll index versus yield in 2008

Year	Location	Variety	Model	Equation	Model (Pr > F)	R ²
2008	GR	KT 204LC	Linear	$y = 23.6x - 1730$	0.0069	0.417
		KT 204LC	Quadratic	$y = -0.397x^2 + 37.6 - 2961$ **	0.0298	0.417
		TN 90LC	Linear	$y = 21.49x - 1447$	0.0058	0.456
		TN 90LC	Quadratic	$y = -0.6199x^2 + 240.55x - 20738$ **	0.0165	0.495
	SP	KT 204LC	Linear	$y = 0.24x + 2571$	0.9847	0.000
		KT 204LC	Quadratic	$y = 2.785x^2 - 925.74 + 79371$	0.3272	0.143
		TN 90LC	Linear	$y = -3.02x + 2951$	0.8003	0.005
		TN 90LC	Quadratic	$y = -1.69x^2 + 556.1x - 43078$	0.5612	0.092
	LE	KT 204LC	Linear	$y = 1.16x + 2469$	0.9185	0.001
		KT 204LC	Quadratic	$y = -1.36x^2 + 470.5x - 37868$	0.4175	0.126
		TN 90LC	Linear	$y = -15.8x + 5166$	0.172	0.129
		TN 90LC	Quadratic	$y = 1.02x^2 - 360.6x + 34154$	0.2131	0.212

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

predicting yield in 2007 and 2008. In 2007, only three models were significant. The quadratic model at SP in 2007 has the highest R^2 value, 0.546, of all the models in 2007. GR was the only site in 2008 that reported significant regression for chlorophyll index values predicting yield. Both the linear and quadratic models for both varieties were significant; however in the quadratic models for both varieties the quadratic coefficient was not significant. Therefore the linear models would be used to predict yield from chlorophyll index values. TN 90LC had a higher R^2 , 0.456, than KT 204LC, 0.417. Figures 4.5 and 4.6 show the linear regression models for KT 204LC and TN 90LC at GR in 2008. With all locations combined the linear and quadratic models were significant. The R^2 values for both equations were very low, 0.048 for the linear model and 0.056 for the quadratic. The quadratic coefficient was not significant. Figure 4.7 is the combined scatter-plot for chlorophyll index explaining relative yield. The linear model is displayed on the graph along with the equation and respective R^2 value. Since the results were inconsistent across sites and the low combined R^2 for the combined model is low, chlorophyll index is not a good predictor of yield at this time during the growing season.

Regression analysis for 5WAT sampling interval

5WAT petiole sap nitrate-N versus yield

At the 5WAT sampling date we saw difference in petiole nitrate-N concentrations between the varying nitrogen rates. The 5WAT petiole nitrate concentrations were paired with their respective yields and regression analyses were conducted using SAS 9.2 to evaluate how well they might predict yields at all locations in 2008. Regression equations for petiole nitrate-N values predicting yield can be found in Table 4.56. A combined analysis was also run for all locations and the equations for this are at the bottom of Table 4.56. Only the regression models

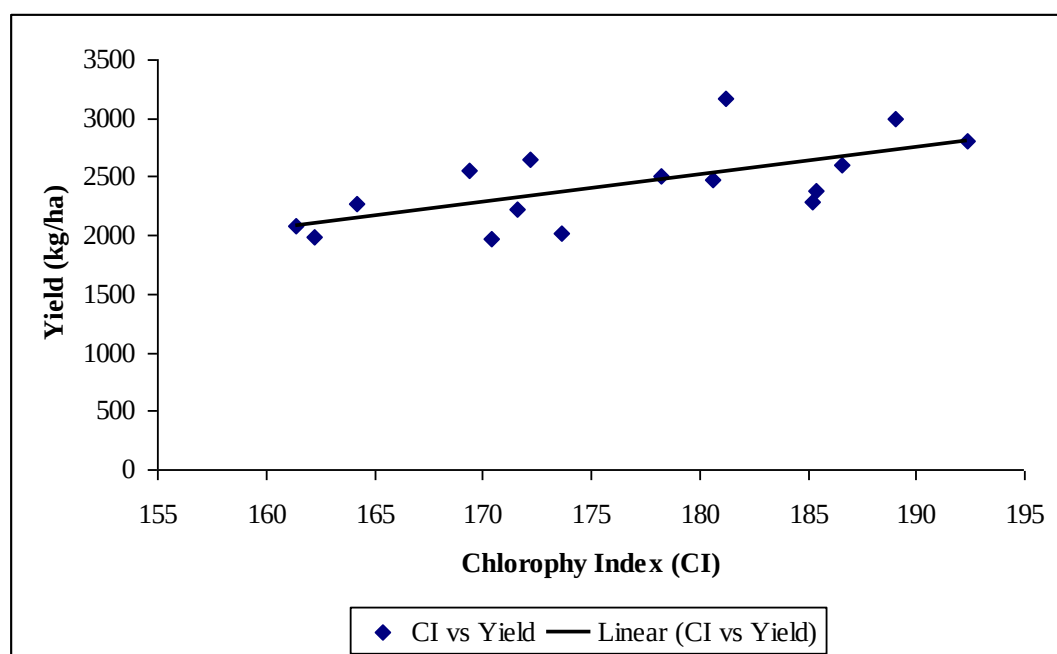


Figure 4.5: Linear regression for KT 204LC chlorophyll index values explaining yield at GR in 2008

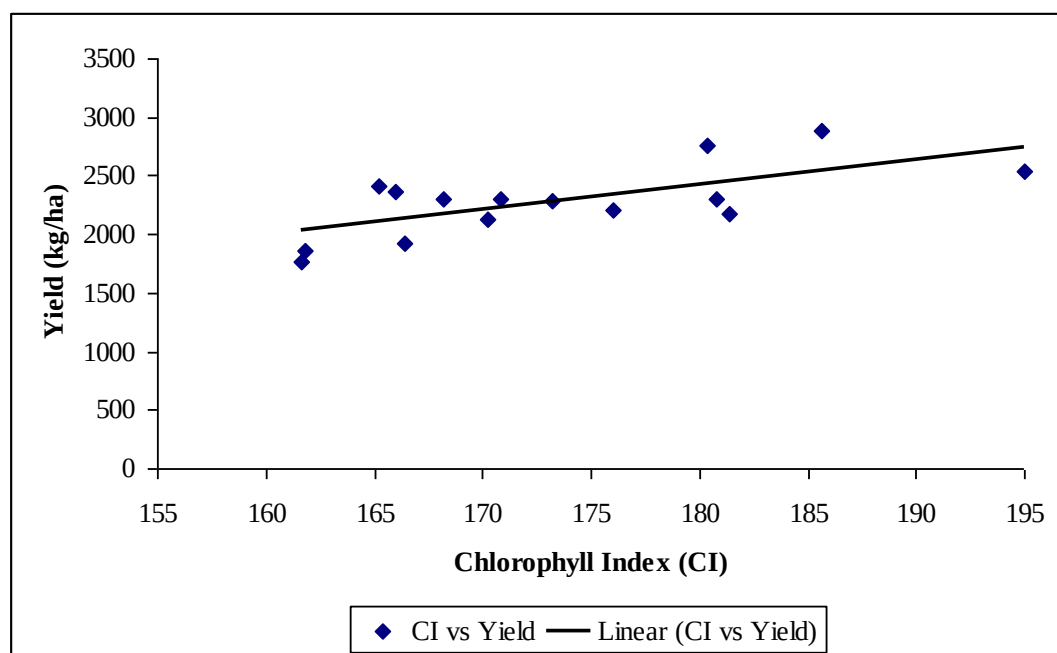


Figure 4.6: Linear regression for TN 90LC chlorophyll index values explaining yield at GR in 2008

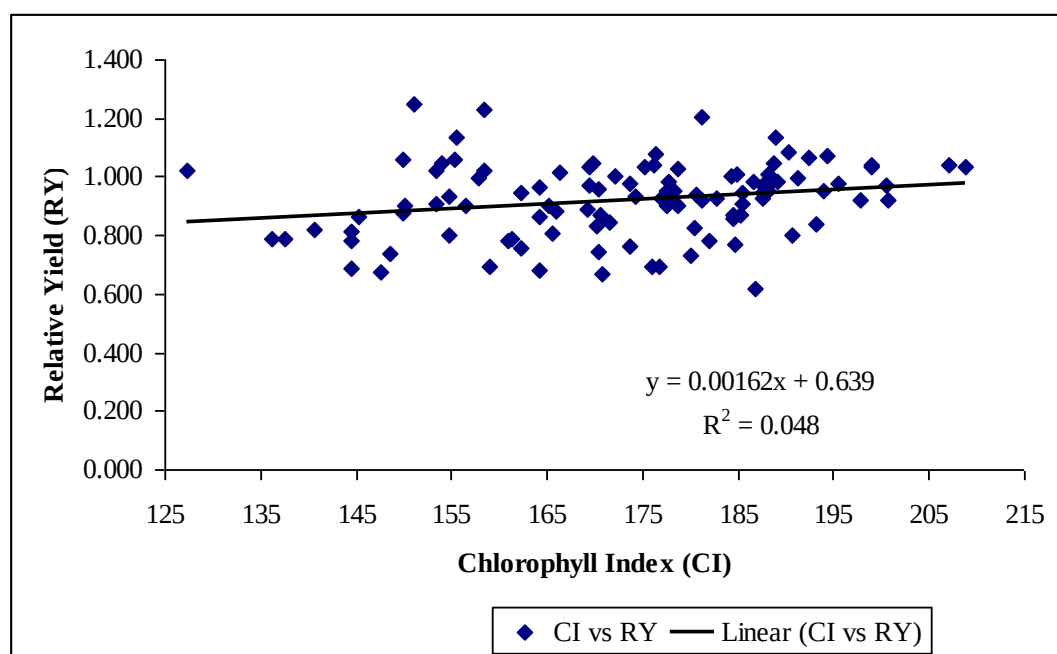


Figure 4.7: Scatter-plot linear equation and R^2 value for the combined KT 204LC chlorophyll index values at all locations

Table 4.56: Regression equations for 5WAT petiole sap nitrate-N concentrations predicting yield in 2008

Year	Location	Variety	Model	Equation	Model	R ²
						(Pr > F)
2008	GR	KT 204LC	Linear	y = -0.475x + 2943	0.3939	0.024
		KT 204LC	Quadratic	y = -0.0016x ² + 2.29x + 1781	0.6427	0.030
		TN 90LC	Linear	y = -0.073x +2469	0.8881	0.001
		TN 90LC	Quadratic	y = -0.00053x ² +0.832x + 2091	0.979	0.002
	SP	KT 204LC	Linear	y = 0.70x + 2043	0.199	0.054
		KT 204LC	Quadratic	y = -0.0023x ² + 5.32x - 270	0.2985	0.080
		TN 90LC	Linear	y = -0.327x + 2871	0.5218	0.014
		TN 90LC	Quadratic	y = 0.0062x ² - 12.9x + 9195	0.1873	0.113
	LE	KT 204LC	Linear	y = 0.34x +1020	0.0246	0.157
		KT 204LC	Quadratic	y = -0.00035x ² + 3.89x - 8040	0.0187	0.240
		TN 90LC	Linear	y = 0.192x + 1675	0.1353	0.073
		TN 90LC	Quadratic	y = -0.00011x ² + 1.23x - 799	0.2781	0.085
All Sites***	KT 204LC	Linear	y = -0.000075x + 0.983	0.5378	0.006	
	KT 204LC	Quadratic	y = 0.0000002x ² – 0.0004x + 1.12	0.7950	0.008	

***Combined analysis used relative yield at the y-variable

for KT 204LC at LE were significant; the R^2 values at this location show a very weak relationship between yield and 5WAT petiole nitrate concentrations. The combined regression models were not significant. Petiole nitrate concentrations at 5WAT were different among nitrogen rates; however, they were not a good predictor of yield. At all sampling dates petiole nitrate concentrations did not accurately correspond to burley tobacco yields, in contrast to results from other crops which show that nitrate-N levels within those crops can be used to manage nitrogen fertilization.

5WAT chlorophyll index versus Yield

At 5WAT, chlorophyll index values showed differences among nitrogen rates at GR but did not show differences at the other sites. GR was the only site at 5WAT that had significant linear and quadratic regression models for both varieties. Table 4.57 contains the regression equations and parameters for all sites and varieties along with the equations for the combined KT 204LC analysis over all sites. The highest reported R^2 values is 0.248 for chlorophyll index values for TN 90LC at GR. The quadratic coefficient for TN 90LC at GR is not significant so the simplest model is the one to use in order to explain yield differences using chlorophyll index. The quadratic models for both varieties at SP are significant for chlorophyll index values. The combined regression equations for KT 204LC were not significant for chlorophyll index and can be found at the bottom of Table 4.57. Chlorophyll measurements have proven useful for predicting nitrogen requirements in other crops; however our results indicate that pre-sidedress and 5WAT chlorophyll index values are not significantly correlated to yield.

Table 4.57: Regression equations for 5WAT chlorophyll index values predicting yield in 2008

Year	Location	Variety	Model	Equation	Model (Pr > F)	R ²
2008	GR	KT 204LC	Linear	$y = 11.9x + 476$	0.0195	0.169
		KT 204LC	Quadratic	$y = -0.144x^2 + 62.3x - 3902$	0.0625	0.174
		TN 90LC	Linear	$y = 16.8x - 582$	0.007	0.248
		TN 90LC	Quadratic	$y = -0.276x^2 + 113.5x - 9016$ **	0.0236	0.259
	SP	KT 204LC	Linear	$y = 6.29x + 1206$	0.1682	0.062
		KT 204LC	Quadratic	$y = 0.455x^2 - 218.9x + 28956$	0.0991	0.147
		TN 90LC	Linear	$y = 6.62x + 980$	0.1039	0.089
		TN 90LC	Quadratic	$y = 0.209x^2 - 94.3x + 13134$	0.089	0.159
	LE	KT 204LC	Linear	$y = 5.69x + 1834$	0.433	0.021
		KT 204LC	Quadratic	$y = -0.81x^2 - 257.1x + 23009$	0.4067	0.060
		TN 90LC	Linear	$y = 2.44x + 2205$	0.7541	0.003
		TN 90LC	Quadratic	$y = 1.32x^2 - 416.6x + 35317$	0.2467	0.092
All Sites***		KT 204LC	Linear	$y = 0.00013x + 0.873$	0.6901	0.002
		KT 204LC	Quadratic	$y = -0.000014x^2 + 0.0059x + 0.311$	0.4980	0.015

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

***Combined analysis used relative yield at the y-variable

Total Nitrogen Applied versus Yield

For regression analysis, preplant and sidedress nitrogen applications were added together to calculate total nitrogen applied. Actual yields were used in the regression analyses at individual locations, while relative yields were used for the combined analysis. Regression equations and parameters for total nitrogen versus yield can be found in Table 4.58 for 2007 and Table 4.59 for 2008. SP was the only model in 2007 that was not significant; this is likely due to the severe drought in 2007 which limited response to nitrogen at this location. However, R^2 values in 2007 do not reflect a strong or even moderate relationship between total nitrogen and yield for the linear or quadratic models. Though SP suffered heavy losses from the drought in 2007, all locations saw lower than normal rainfall and this could explain the low correlation between total nitrogen applied and yield. The highest R^2 value for regression at individual locations was 0.261 for the linear model at GR. The linear model is the most appropriate, even though the quadratic model R^2 for GR is higher, because the quadratic coefficient was not significant.

In 2008, the weather was more favorable and the relationship between total nitrogen applied and yield at individual locations became apparent. GR was the only location where total nitrogen was not highly correlated to yield. GR in 2008 was under disease pressure, as mentioned in the yield section previously, from black shank and this may have been partially responsible for the limited N response. SP had R^2 values for KT 204LC of 0.552 for the linear model and 0.628 for the quadratic model. For TN 90LC the R^2 values were 0.323 for the linear model and 0.337 for the quadratic model at SP. At LE R^2 values were above 0.619 for all varieties and models. With the exception of GR, total nitrogen was a good indicator of yield

Table 4.58: Regression equations for total nitrogen applied versus yield at all locations in 2007

Year	Location	Model	Equation	Model (Pr > F)	R ²
2007	GR	Linear	$y = 1.485x + 3644$	0.0008	0.261
		Quadratic	$y = -0.0041x^2 + 2.726x + 3585$ **	0.0025	0.277
	SP	Linear	$y = 0.403x + 2014$	0.572	0.008
		Quadratic	$y = -0.011x^2 + 3.63x + 1859$	0.363	0.053
	GL	Linear	$y = 1.65x + 3659$	0.0058	0.184
		Quadratic	$y = -0.011x^2 + 5.038x + 3493$	0.0046	0.253
	LE	Linear	$y = 1.38x + 3762$	0.0095	0.164
		Quadratic	$y = -0.0066x^2 + 3.35x + 3667$ **	0.0189	0.193
	DS	Linear	$y = 1.14x + 2718$	0.0193	0.136
		Quadratic	$y = -0.0046x^2 + 2.53x + 2652$ **	0.0467	0.153

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

Table 4.59: Regression equations for Total nitrogen applied versus yield at all locations in 2008

Year	Location	Variety	Model	Equation	Model (Pr > F)	R ²
2008	GR	KT 204LC	Linear	$y = 0.961x + 2412$	0.2259	0.049
		KT 204LC	Quadratic	$y = -0.0015x^2 + 1.32x + 2399$	0.4817	0.049
		TN 90LC	Linear	$y = 1.22x + 2259$	0.0941	0.104
		TN 90LC	Quadratic	$y = -0.002x^2 + 1.70x + 2242$	0.248	0.105
	SP	KT 204LC	Linear	$y = 3.42x + 2326$	0.0001	0.552
		KT 204LC	Quadratic	$y = -0.017x^2 + 7.56x + 2170$	0.0001	0.628
		TN 90LC	Linear	$y = 2.72x + 2205$	0.0008	0.323
		TN 90LC	Quadratic	$y = -0.0078x^2 + 4.65x + 2126$ **	0.0031	0.337
	LE	KT 204LC	Linear	$y = 4.14x + 2247$	0.0001	0.615
		KT 204LC	Quadratic	$y = -0.0083x^2 + 6.13x + 2172$ **	0.0001	0.628
		TN 90LC	Linear	$y = 4.61x + 2039$	0.0001	0.619
		TN 90LC	Quadratic	$y = -0.0022x^2 + 5.15x + 2019$	0.0001	0.620

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

when environment conditions were favorable for burley tobacco production.

When total nitrogen versus yield data was analyzed across all locations, absolute yield was converted to relative yield to normalize the data so that individual site yield potentials would not affect the curve. When viewed as a scatter-plot the data reached a plateau, so the combined analysis was run using the quadratic plateau model in the NLIN procedure in SAS 9.2. The quadratic plateau model was significant and Figure 4.8 shows the relationship along with the quadratic equation and join point. Relative yields reached a plateau of 0.953 with 204.8 kg/ha total nitrogen. This critical nitrogen rate falls in the middle of the range of recommended nitrogen for burley tobacco in the major burley tobacco producing states. In the ANOVA for yields, there was no response seen above 135 kg/ha total nitrogen when all sites were combined. The results of the mean separation conflict with the quadratic plateau model (Figure 4.8). One reason for this is the difference in relative yield between 204.8 and 135 kg N/ha rate in the model is not greatly different. Burley tobacco is a high value crop and a difference of 100-200 kg/ha in yield results in a considerable amount of income; this will often exceed the cost of the extra nitrogen that needs to be applied in order to reach maximum yields.

Total Nitrogen applied versus Total TSNA content

TSNA samples were taken from each plot after curing and sent to the University of Kentucky for analysis. Total TSNA content has been correlated to nitrogen fertilization in previous research (MacKown et al., 1999; MacKown and Sutton, 1997; Burns, 1986; Marchetti et al., 2006; Atkinson and Sims, 1973; Aycock et al., 1979, Jones and Tramel, 1979; Broaddus et al., 1965; Hamilton et al., 1982; Elliot and Court, 1978; McKee, 1978; Link and Terrill, 1982; Bush et al., 2001; Wahlberg et al., 1999; Wiernik et al., 1995; Hamilton et al., 1982; Rundolf et

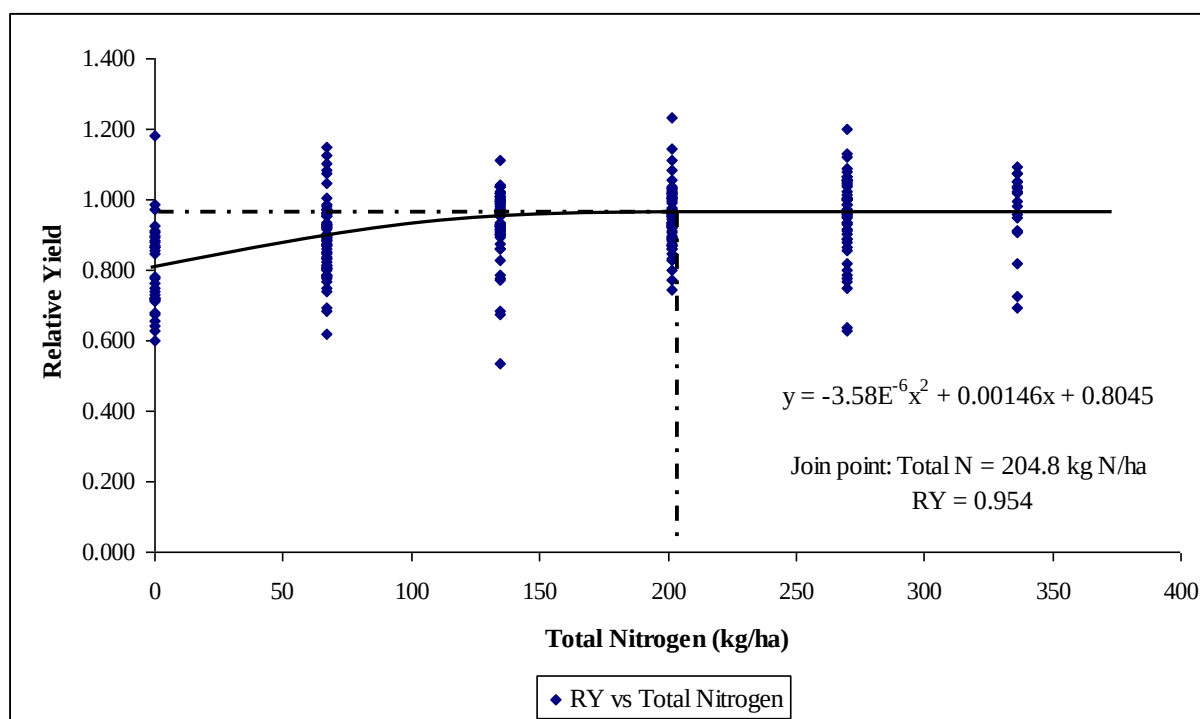


Figure 4.8: Quadratic plateau model for Total nitrogen applied versus relative yield all locations combined

al., 2000). Regression models looking at the correlation between total nitrogen and total TSNA content can be found in Table 4.60. TSNA data was only available for 2007 so no regression models are presented for 2008. GR had the highest R^2 values for total nitrogen predicting TSNA content in the cured leaf. SP had the lowest R^2 values of any of the sites and was the only site with a regression model that was not significant. The drought played a significant role in TSNA formation at all locations, but at SP the low correlations between TSNA content and total nitrogen compared to the other locations is likely due to the drought. The above equations and R^2 values show that there is a moderate correlation between TSNA content and total nitrogen applied.

When all data were combined, total TSNA content was converted to a relative TSNA content (RT) because curing conditions at each of the individual locations were different and one site had total TSNA contents 2-3 times higher than the other locations. RT was used to normalize TSNA content for each location so regression analysis could be run. A scatter-plot was made of RT versus total nitrogen and a plateau was seen in the combined data. The

Table 4.60: Total nitrogen applied versus total TSNA content in 2007

Year	Location	Model	Equation	Model (Pr > F)	R^2
2007	GR	Linear	$y = 0.0035x + 0.458$	0.0001	0.573
		Quadratic	$y = -0.0000077x^2 + 0.0058x + 0.348$ **	0.0001	0.595
	SP	Linear	$y = 0.0009x + 1.128$	0.11	0.066
		Quadratic	$y = -0.000012x^2 + 0.0045x + 0.952$	0.042	0.158
	GL	Linear	$y = 0.007x + 3.25$	0.0001	0.341
		Quadratic	$y = -0.000012x^2 + 0.011x + 3.061$ **	0.0003	0.35
	LE	Linear	$y = 0.003x + 0.816$	0.0002	0.312
		Quadratic	$y = -0.000016x^2 + 0.0077x + 0.579$	0.0001	0.393

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

combined data was then analyzed using the quadratic plateau model in the NLIN procedure in SAS 9.2. Figure 4.9 is the graph of the quadratic plateau model for total TSNA versus total nitrogen applied. The plateau for RT was at 0.877 at 257.8 kg/ha total nitrogen applied. Previous research by Waynick (2007) has shown that TSNA content maintains a linear relationship as nitrogen rates increase, which is not what is seen in Figure 4.9. One explanation for the plateau seen in 2007 is that during the yellowing phase of the curing process there were high temperatures and low humidity. This combination resulted in tobacco that dried quickly, perhaps not allowing the bacteria, which reduce nitrate to nitrite, to continue nitrate reduction that feeds the TSNA formation reaction. So, a RT plateau was seen as a result of the curing conditions in 2007. Also, the optimum nitrogen rate of 204.8 kg/ha total nitrogen, to maximize yields, is well below the total nitrogen rate, 257.8 kg N/ha, at the plateau for relative TSNA content seen in the model.

Petiole Nitrate at all sampling intervals versus Total TSNA content

Since nitrate is a precursor to the nitrite needed in the nitrosation reactions which produce TSNA in the cured leaf of tobacco, the petiole nitrate-N concentrations that were measured at multiple dates within the growing season were evaluated for their ability to predict TSNA content in cured leaf. Nitrite is considered to be a better predictor of TSNA than nitrate, but plants do not accumulate high concentrations of nitrite because it is toxic. Table 4.61 contains the regression analysis for the sampling intervals and each location in 2007. At the pre-sidedress sampling interval only one model was significant for petiole nitrate predicting total TSNA content. The quadratic model at GR for the pre-sidedress sampling interval has an R^2 value

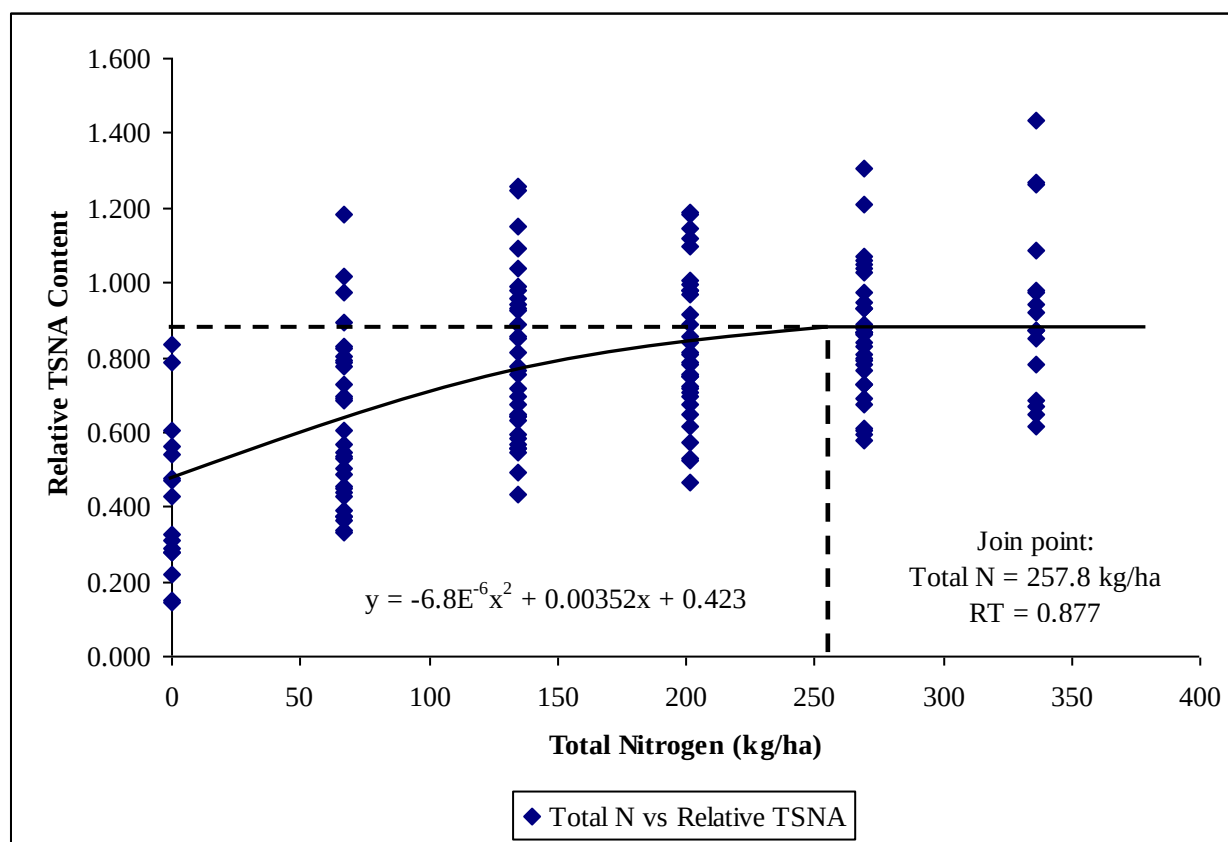


Figure 4.9: Quadratic plateau model for total TSNA content versus total nitrogen applied for all locations combined in 2007

Table 4.61: Petiole nitrate concentrations versus total TSNA content at all sampling dates and locations in 2007

Sampling Date	Location	Model	Equation	Model Pr > F	R ²
Pre-sidedress	GR	Linear	$y = 0.000083x + 0.748$	0.8388	0.0024
		Quadratic	$y = -0.0000026x^2 + 0.0077x - 4.72$ *	0.0394	0.317
	SP	Linear	$y = 0.00033x + 0.803$	0.1812	0.097
		Quadratic	$y = -0.00000092x^2 + 0.0022x - 0.276$	0.1336	0.211
	GL	Linear	$y = 0.002x + 1.55$	0.1012	0.142
		Quadratic	$y = -0.000006x^2 + 0.016x - 0.683$	0.1622	0.193
Pre-topping	GR	Linear	$y = 0.002x - 0.875$	0.0001	0.332
		Quadratic	$y = -0.00000063x^2 + 0.0032x - 1.409$ **	0.0006	0.333
	SP	Linear	$y = 0.0002x + 1.094$	0.118	0.063
		Quadratic	$y = 0.00000013x^2 - 0.000039x + 1.189$	0.278	0.067
	GL	Linear	$y = 0.0012x + 3.22$	0.066	0.086
		Quadratic	$y = -0.000002x^2 + 0.005x + 1.29$ **	0.099	0.117
Pre-Harvest	GR	Linear	$y = 0.0074x + 0.092$	0.0001	0.508
		Quadratic	$y = 0.000000039x^2 + 0.0008x + 0.049$ **	0.0001	0.508
	SP	Linear	$y = 0.0003x + 0.928$	0.051	0.097
		Quadratic	$y = -0.00000048x^2 + 0.0013x - 0.478$ **	0.083	0.126
	GL	Linear	$y = 0.0013x + 2.38$	0.011	0.156
		Quadratic	$y = -0.000003x^2 + 0.0099x - 3.163$	0.0057	0.244

* Linear term is not significant at alpha = 0.1 level of probability when model was significant

** Quadratic term is not significant at alpha = 0.1 level of probability when model was significant

0.317. Because most of the models at the pre-sidedress sampling interval are not significant and the low R^2 values, petioles nitrate-N concentrations at this point during the growing season are not a good predictor of total TSNA content in the cured leaf.

Regression models at pre-topping for GL and GR are significant, while the regression models for SP are not significant. At GR the quadratic coefficient is not significant therefore the linear model is the most appropriate model for the location and has an R^2 value of 0.332. This is the highest R^2 for any regression model at the pre-topping sampling interval. Petiole nitrate-N at the pre-topping sampling interval does not have a strong correlation to total TSNA content and would not be a good predictor of TSNA content.

At the pre-harvest sampling interval all models for all locations were significant. The linear model at GR had the highest R^2 value, 0.508 at this interval. The next highest R^2 value was at GL for the quadratic model and was 0.244. Though all the models at each location were significant the relationships between total TSNA and petiole nitrate-N concentrations were not strong enough to make reliable predictions about TSNA content.

As the sampling intervals approached harvest, the relationship between total TSNA content and petiole nitrate-N levels strengthened. Models with the predictive ability of the pre-harvest model at GR with a R^2 of 0.508 would be needed at all locations to have the potential to use petiole nitrate-N at harvest for TSNA prediction. However, the weather throughout the growing season as well as during the curing season may have played a part in the poor correlation between total TSNA and petiole nitrate-N levels at the three sampling intervals. Higher temperatures and low humidity during the first few weeks of curing could have depressed TSNA levels in the cured leaf by inhibiting microbial activity during the yellowing phase of the curing process.

Chapter 5

Conclusions and Recommendations

Yield

Burley tobacco has always been considered a crop that needs high rates of nitrogen in order to maximize yield. The philosophy has always been that nitrogen fertilizer was cheap compared to the yield gains from added nitrogen to burley tobacco. In this study the goal was to evaluate burley tobacco over nitrogen rates that ranged from 0 kg N/ha to 336 kg N/ha in 2007 and from 0 to 270 kg/ha in 2008. Unlike the previous study conducted by Waynick (2007), a 0 kg/ha total nitrogen treatment was added as a control or check plot. The forty year average for burley tobacco yields in Tennessee is 2,197 kg/ha (Waynick, 2007). At all sites, except SP in 2007, over both years, 67 kg/ha total nitrogen rate produced higher yields than the forty year average for Tennessee. When yields for all sites were combined, the 0 kg/ha total nitrogen rate had an average yield of 2,713 kg/ha. The potential to reduce nitrogen fertilizer use is apparent from this data. However, the fields that were in this study, with one exception, were in three year rotations which help break disease cycles and maintain soil fertility levels as compared to fields that are in continuous tobacco production. Optimum yields, defined as yields not statistically different than the maximum yields, were reached with 135 kg/ha total nitrogen when all sites from both years were combined. This was true for both KT 204LC and TN 90LC at all sites. There was no benefit to split application of nitrogen fertilizer at any of the locations. Yields were the same if 135 kg N/ha was put out all preplant or split into 67 kg N/ha preplant + 67 kg N/ha sidedress. The 135 kg/ha total nitrogen at which yields were optimized is lower than the current recommendations for the leading burley tobacco producing states. Another aspect of this

study looked at four different varieties across five total nitrogen rates in 2008 only. In this portion of the study NC 7 and KT 204LC consistently had higher yields than TN 90LC and TN 90HC over all nitrogen rates, but nitrogen response did not differ between varieties. In the combined analysis for all varieties, optimal yields were reached at the 202 kg/ha total nitrogen rate which is in the range of nitrogen fertilization recommendations for the leading burley tobacco producing states. When looking at the relationship between total nitrogen applied versus relative yield using a plateau regression model, a critical value of 204.8 kg N/ha was established to achieve relative yields of 0.954. This critical total nitrogen rate falls within the recommended nitrogen fertilization rates for all major burley tobacco producing states. This means at this critical total nitrogen rate, 95.4% of the highest average yield for that location will be achieved. Out of all plant and soil monitoring techniques used in this study, total nitrogen fertilizer applied was the best tool to use for predicting yields. Based on the data from this study, the current recommended nitrogen rates look adequate in order to optimize yields for the commercial grower.

PSNT

The PSNT has been very successful in crops such as corn and other specialty crops, but in previous research the PSNT was not found to be the best predictor of yield. In this study the PSNT differed by preplant nitrogen rates at all sites. PSNT values increased as nitrogen rates increased. When all sites were combined and plotted against relative yield (Figure 4.3) there was a clear plateau with no yield increase beyond a certain PSNT level. The quadratic plateau model was used to analyze the dataset and a critical PSNT value was calculated. The critical level was 46.4 ppm soil nitrate-N at a relative yield of 0.946. This critical PSNT value is reached in the

combined analysis at the 67 kg N/ha preplant treatment (Table 4.15). We know that yields were not optimized in the combined analysis until 135 kg/ha total nitrogen was applied, which contradicts the critical PSNT results. The total nitrogen quadratic plateau model had a critical level at 204.8 kg N/ha which also conflicts with the PSNT critical level. The PSNT critical level is within the same range as previous studies on burley tobacco. However, given the other results for yield in this study, the PSNT critical level may be too low. One cause for this is there are multiple soil types being grouped together in the combined analysis, as well as different rotation practices that can alter soil nitrate levels. Figure 4.3 shows that there is a plateau, but more precise work on individual soil types may make this a potential tool for nitrogen recommendations in burley tobacco in the future. MacKown (1999) set two different critical PSNT levels on two separate soil types for burley tobacco, and this approach may be what is needed in order to use this test as a tool. The critical level found in this study is consistent with prior research in burley tobacco, in which burley tobacco has a higher critical PSNT value than those associated with corn and other crops.

Petiole Nitrate (Cardy® Nitrate Meter)

Petiole nitrate concentrations have been used in specialty crops as a way of monitoring plant nitrogen status during the growing season. Some crops even have nitrogen fertilization recommendations based on petiole nitrate concentrations. In this study petiole sap nitrate-N concentrations did increase with increasing nitrogen rates. LE in 2008 had much higher petiole sap nitrate-N concentrations than any other site. The data for LE was not incorporated into the combined analysis and regression model explaining yield. The 0 kg/ha total nitrogen treatments decreased in petiole nitrate concentrations throughout the growing season. All other nitrogen

rates decreased between the pre-sidedress interval and pre-topping interval, but from the pre-topping interval petiole nitrate concentrations increased in the plant. At the pre-harvest interval in 2008, KT 204LC, TN 90LC and TN 90LC were sampled for petiole sap nitrate-N. Each variety received five total nitrogen rates and petiole nitrate-N concentrations increased up to 202 kg/ha total nitrogen applied, above which no increase in nitrate concentration was seen. In the combined analysis KT 204LC had significantly less petiole nitrate-N concentrations than TN 90LC and TN 90HC over all nitrogen rates.

Though differences were seen at all sampling dates in the combined analysis, when plotted against yield, petiole nitrate concentrations were a poor predictor of yield. Regression analyses were conducted pre-sidedress and 5WAT because these were the intervals that still allowed for adjustments in nitrogen fertilization if yield could be predicted by petiole nitrate concentrations. At 5WAT petiole nitrate concentrations did not improve in the ability to predict yield. Changing the leaves which are sampled may increase the chance of being able to correlate petiole nitrate-N to yield in order to make nitrogen fertilization recommendations. Sampling the lower leaves may increase the chances of finding larger differences since there are higher concentrations of nitrate in the lower stalk positions in burley tobacco. Also, young leaves were sampled in this study; older mature leaves may show larger differences because of translocation of nutrients within the plant to areas of new growth.

Leaf Chlorophyll Content (CM 1000® chlorophyll meter)

Nondestructive chlorophyll measurements are a relatively new tool to use to evaluate crop nitrogen status in the field. Chlorophyll is directly related to nitrogen content of the plant and a green looking plant is usually considered a healthier plant than one that is chlorotic.

Chlorophyll index values were inconsistent across all location and sampling intervals.

Differences in chlorophyll index values were detected at only a few locations, and the differences were so small it would be difficult to make nitrogen fertilization recommendations unless a high number of samples were taken. The combined analysis did show differences at the pre-topping sampling interval; however this is too late in the season to adjust nitrogen rates in hopes to improve or limit yield loss. In crops where chlorophyll meters have been used successfully, most of the measurements have been on crop canopy not individual leaves. Variation from plant to plant can be great within a field, and this is true on a leaf to leaf basis. When plotted against yield, chlorophyll index values were poor indicators of yield at almost every location. GR in 2008 had the highest R^2 value of all the chlorophyll index regression equations predicting yield (Table 4.55). Overall, more work needs to be done using chlorophyll index values for the evaluation of nitrogen status in burley tobacco, as well as predicting yield.

TSNA

TSNA data were only available for 2007 for this study. As in previous research, TSNA content increased as nitrogen fertilization increased. The individual TSNA also increased with increasing nitrogen rates. NAT was the predominant TSNA present in cured leaf samples, followed by NNN. 2007 was a great year for reduced TSNA content in cured leaf, but a horrible year for curing high quality tobacco. The hot and dry curing season resulted in low TSNA content in cured leaf. TSNA concentrations were less than $3\mu\text{g/g}$ for all nitrogen treatments for the combined analysis. GL was the only individual site where TSNA concentrations went above $2\mu\text{g/g}$ for any nitrogen treatment. GL has previously been much higher in TSNA content than

other locations; microclimate at the curing site is thought to be the reason why higher TSNA content is seen at this location.

Since nitrate is involved in the TSNA formation process, petiole nitrate concentrations were analyzed to evaluate if they could accurately predict total TSNA content. At the pre-sidedress sampling interval only one regression model was significant. This was at GR which had the most consistent trend in R^2 values for petiole nitrate-N predicting total TSNA content. The R^2 values for the regression models for GR increased at each sampling interval during the growing season. All models at each location were significant at the pre-harvest interval, but other than GR none have a strong relationship for predicting total TSNA content. In addition to petiole nitrate-N concentrations, total nitrogen applied was correlated to total TSNA content. All locations have higher R^2 values for total N applied as a predictor of total TSNA content than the petiole nitrate-N concentrations at various sampling intervals. SP was the only site where the linear model for total nitrogen applied was not significant. This is most likely due to the variability caused by the drought. When total nitrogen versus total TSNA data was combined the scatter-plot showed a plateau. Figure 4.9 shows the quadratic plateau model for total nitrogen versus total TSNA content. Total TSNA values were normalized using the same method that was used for relative yield. The figure shows TSNA formation reaching a plateau at 257.8 kg/ha total nitrogen at 0.877 relative TSNA. This level of nitrogen fertilization is outside the recommended rates for Tennessee, so therefore in a year like 2007 producers will not have to worry about maximizing TSNA content. TSNA usually increased linearly with increasing nitrogen fertilizer, however the bad curing season may have pushed the tobacco through yellowing quicker than in a normal curing season resulting in less time for TSNA to form and ultimately lowering TSNA content across all sites. TSNA formation is a biological reaction and

is influenced by many factors, however this study shows that TSNA content does increase with increasing nitrogen fertilization.

Overall yields do increase as more nitrogen is applied and this study has shown that the current nitrogen recommendations for burley tobacco in the major producing states are adequate to attain optimum yield. In fact, data shows that the higher end of these recommendations may be more than adequate. Total nitrogen is the best predictor of yield and TSNA content from the results of this study. However, TSNA data is not available for 2008 and with more rainfall than 2007 petiole nitrate-N concentrations may be more closely related to TSNA. PSNT has the potential for being a good predictor of yield for burley tobacco; however more data needs to be collected based on soil type and yield potential to determine critical PSNT levels. The chlorophyll meter and Cardy® meter proved not to be predictors of yields early enough in the season to allow addition of supplemental fertilizer if needed in burley tobacco.

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Appendix

Table A.1: 2007 Grade Index for all nitrogen rates

Preplant N	Sidedress N	GR	SP
kg/ha	kg/ha	Grade Index	
0	0	55.9	30.9
0	67	63.2	34.6
67	0	58.6	42.4
67	67	63.5	30.5
135	0	60	39.3
135	67	63.5	33.1
202	0	58.6	32.8
202	67	57.1	36.4
270	0	59.6	33.4
270	67	62.9	32.9

* Values with different letters within the same location are significantly different at 0.1 level of probability

Table A.2: Grade Index for all varieties and total nitrogen in 2008

Location	Variety	Total Nitrogen Applied					Average
		0	67	135	202	270	Gr. Index
----- kg/ha -----							
GR	KT 204LC	49.4	62.3	47.6	47.2	58.5	53
	TN 90LC	58.4	54.3	45.8	57.1	61	55.3
	TN 90HC	41.6	56.9	43.4	49.8	52.1	48.7
	NC 7	54.1	63.5	46.4	54.4	53	54.2
Average	Gr. Index	50.8	59.3	45.8	52.1	56.1	
SP	KT 204LC	33.0 bc*	52.8 abc	61.5 a	62.0 a	44.3 abc	50.7 B
	TN 90LC	29.1 c	45.8 abc	53.3 abc	57.0 abc	47.3 abc	46.5 B
	TN 90HC	33.3 bc	42.5 abc	52.0 abc	53.0 abc	48.5 abc	45.9 B
	NC 7	46.3 abc	62.3 a	58.0 ab	67.0 a	56.3 abc	58.6 A
Average	Gr. Index	35.4	51.6	56.2	59.8	49.1	

* Mean comparisons are within locations. Main effect means followed by the same capital letter are not significantly different at $p=0.10$. Variety by N rate means followed by the same lower case letter are not significantly different at $p=0.10$.

Table A.3: 2007 and 2008 Weather Data for GR

2007	Avg. Daily.	Dept. from	Avg. Daily.	Dept. from	Total	Dept. from
	Max Temp.	Normal	Min Temp	Normal	Precip.	Normal
Month	°F				in	
May	82	2	50	-3	1.15	-3.32
June	85	-2	58	-3	2.98	-1.24
July	85	-4	63	-1	5.61	0.88
August	94	6	66	7	0.59	-3.21
September	87	4	57	0	1.47	-1.78
October	78	5	49	4	1.38	-0.97
2008						
May	-*	-	-	-	4.27	-0.20
June	-	-	-	-	2.39	-1.83
July	-	-	-	-	3.98	-0.75
August	-	-	-	-	3.87	0.07
September	-	-	-	-	2.19	-1.06
October	-	-	-	-	1.84	-0.51

* Data not reported

Table A.4: 2007 and 2008 Weather Data for LE

	Avg. Daily.	Dept. from	Avg. Daily.	Dept. from	Total	Dept. from
	Max					
2007	Temp.	Normal	Min Temp	Normal	Precip.	Normal
Month	°F				in	
May	79	3	56	1	1.45	-3.02
June	84	1	63	1	1.77	-1.89
July	93	7	64	-1	6.90	1.90
August	90	6	69	6	2.56	-1.37
September	84	6	60	5	1.15	-2.08
October	73	5	53	8	5.28	2.71
2008						
May	71	-5	52	-3	4.88	0.41
June	84	1	64	2	3.30	-0.36
July	87	1	65	0	2.54	-2.46
August	87	3	63	0	1.08	-2.85
September	84	6	60	5	1.21	-3.23
October	70	2	45	0	1.35	-1.22

Table A.5: 2007 and 2008 Weather Data for SP

	Avg. Daily.	Dept. from	Avg. Daily.	Dept. from	Total	Dept. from
2007	Max Temp.	Normal	Min Temp	Normal	Precip.	Normal
Month	°F				in	
May	83	4	55	1	3.65	-1.73
June	89	4	61	-1	2.20	-2.58
July	90	3	64	0	1.61	-2.57
August	100	15	68	6	1.16	-2.57
September	87	8	59	3	2.53	-1.15
October	77	8	50	4	6.24	3.06
2008						
May	75	-4	53	-1	5.92	0.54
June	87	2	66	4	1.81	-2.97
July	89	2	67	3	5.83	1.65
August	86	1	65	3	1.52	-2.21
September	83	4	61	5	1.93	-1.75
October	71	2	47	1	4.44	1.26

Vita

William Hunter Frame was born on June 3, 1985 in Powhatan County, Virginia. The author is the son of William Michael Frame and Debra O. Zacharias. He attended Powhatan High School and graduated in 2003. William entered Virginia Tech in the fall of 2003, in Blacksburg, VA. He graduated from Virginia Tech in spring of 2007 with a Bachelors of Science in Crop and Soil Environmental Sciences.

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