ABSTRACT

HOLT, JOHN SETH. Using Cotton Leaf and Petiole Tissue to Assess Nitrogen, Phosphorous, and Potassium Interactions within the Cotton Plant. (Under the Direction of Drs. Keith Edmisten and Randy Wells).

With the ever-rising cost in inputs including fertilizer, fuel, pesticides, and seed cotton producers are looking to optimize the use of all agronomic inputs. Agricultural practices have been developed and improved to maximize the efficiency of fertilizer use in cotton (*Gossypium hirsutum*). Innovative technologies such as leaf and petiole tissue testing have allowed cotton growers to monitor plant nutritional status throughout the growing season. Levels of total nitrogen, potassium, phosphorous, and nitrate in the leaf and petiole tissue are commonly used to assess the nutritional needs of the plant, resulting in the grower being able to amend a nutritional deficiency during the growing season.

Using leaf and petiole tissue on broadleaf plants has proven to be a useful and quick tool for assessing plant nutrient deficiencies. Soil sampling for nutrient deficiencies ahead of planting has long been a valuable agronomic practice; however with large rainfall events throughout the growing season that may possibly leach nitrogen and potassium, soil samples are unable to compete with the speed and turnaround of tissue analysis. Field studies were conducted to determine the correlation among available nutrients within leaf and petiole tissue.

A three year study was conducted at the Upper Coastal Plain Research Station in Rocky Mount, North Carolina with variable rates of nitrogen and potassium fertilizer applied at match head square in a complete randomized block design. Nitrogen was applied at 0, 45, 90, and 134 kg/ha^{-1}. Potassium was applied at 0, 56, and 112 kg/ha^{-1}. Leaf and petiole tissue

samples were collected once weekly for 8 consecutive weeks beginning at match head square.

Data collected from both leaf and petiole tissue samples suggested that lower rates and availability of nitrogen significantly affect the amount of potassium uptake. Higher nitrogen rates (90 and 134 kg/ha^{-1}) always led to an increase in concentration of leaf and petiole potassium. Lower rates and availability resulted in higher nitrogen and nitrate concentrations in petiole tissue. Petiole nitrogen, potassium, and nitrates directly followed precipitation events, with rises in petiole nutrient concentrations within 7 days of rainfall. The data collected in all three years revealed that nutrient concentrations in leaf and petiole tissue are co-dependent on one another and can be utilized to make and withhold applying a fertilizer amendment during the growing season.

Using Cotton Leaf and Petiole Tissue to Assess Nitrogen, Phosphorous, and Potassium Interactions within the Cotton Plant

by
John Seth Holt

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APPROVED BY:

Keith Edmisten
Committee Co-Chair

Randy Wells
Committee Co-Chair

Carl Crozier
Committee member

DEDICATION

This dissertation is dedicated to my parents, Johnny and Lynne Holt, and sister, Jessica who have always supported and encouraged me throughout all of my endeavors. Also, to all of my professors and graduate colleagues who helped me achieve this degree, I thank you all. Above all, I give thanks and praise to God and my lord and savior Jesus Christ who made it possible for me to earn a Master of Science.

BIOGRAPHY

John Seth Holt was born on 1 May 1987 in Pinehurst, North Carolina. He is the younger of two children born to Johnny and Lynne Holt. Seth received his elementary and secondary education in Sanford, NC, graduating from Lee County High School in 2005.

Seth grew up on a tobacco farm in White Hill, NC. While working on his father's tobacco farm he also helped neighbors to grow and harvest their tobacco crops. Seth was very actively involved in FFA throughout high school serving as President of the Lee County High School FFA Chapter and the North Carolina Central Region Vice President.

He received his bachelor's degree in 2009 in Agronomy with a focus in Crop Production from North Carolina State University. During his undergraduate education, Seth served as President of the North Carolina State University Agronomy Club, 2008. After graduating, the author began pursuing a Master of Science, Crop Science at North Carolina State University under the direction of Dr. Keith Edmisten, Dr. Randy Wells, and Dr. Carl Crozier. While pursuing his degree, Seth assumed the position of Agriculture Extension Agent in Lee County in 2010 through 2012. After serving as Agriculture Extension Agent, Seth accepted a position with Crop Production Services as a Crops Consultant in February 2012, and continues to serve in this position today. Seth has also returned to his family farm where he and his father now raise beef cattle and commercial produce. In the winter of 2015, Seth officially completed a Master of Science in Crop Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Literature Review of using Cotton Leaf and Petiole Tissue to Assess Nitrogen, Phosphorous, and Potassium Interactions within the Cotton Plant.

John Seth Holt

J.S. Holt, Dept. of Crop Science, North Carolina State University,

Email: jsholt@ncsu.edu, Phone (919) 770-9669

Abbreviations: N, nitrogen; P, phosphorous; K, potassium; NO_3^-, nitrate nitrogen; NRA, nitrate reductase activity

INTRODUCTION

Upland cotton (*Gossypium hirsutum*) has long been an important economic agricultural commodity in North Carolina. Within the Gossypium genus, two of the five tetraploid species (2n = 4x = 52; G. hirstum and G. barbadense), along with two diploid species (2n = 2x =26; G. herbaceum and G. arboreum), have been domesticated over thousands of years (Brubaker et al. 1999). Upland cotton currently dominates world cotton cultivation and provides over 90 percent of the annual cotton crop, has spread from its original home in Mesoamerica to over forty countries (Brubaker et al., 1999). Under domestication, humans have been able to manipulate a perennial shrub into plants that have annual plant characteristics with larger seeds bearing longer, thicker and stronger cellulose fibers.

The cotton plant is a perennial plant with an indeterminate growth habit and is reputed to have the most complex structure of any major field crop (Mauney, 1984). Indeterminate growth characteristics ensure that vegetative and reproductive growth are occurring simultaneously, thereby increasing the difficulty of efficiently managing production practices such as fertilizer application and irrigation (Oosterhuis and Jernstedt, 1999). To properly design and implement crop-management programs that would enhance these factors, the anatomy and morphological development of the cotton plant must be clearly understood (Oosterhuis and Jernstedt, 1999). True leaves arise from the main stem (main-stem leaves) and from the sympodial/fruiting branches (Oosterhuis and Jernstedt, 1999). The main-stem leaves mainly contribute to vegetative development (Oosterhuis and Urwiler, 1988), but also to boll development (Brown, 1968; Wullschleger and Oosterhuis, 1990). On the other hand, sympodial leaves are almost exclusively associated with carbon partitioning to the boll (Constable and Rawson, 1980; Ashley, 1972). Leaf physiological factors can be major assessment tools for determining crop yield. The stature of the plant canopy will determine its ability to intercept solar radiation (Wells, 1991). The canopy is comprised of leaves and their supporting stems and petioles. The leaf utilizes the absorbed solar radiation to drive photosynthetic metabolism and is the conduit through which transpiration occurs thus allowing plant temperature control. In addition leaves act as a site of nutrient assimilation (Cothren 1999). Cotton plants require the primary (N, P, and K) nutrients to drive growth and development (Silvertooth et al. 1999). Monitoring cotton N, P, K, and NO_3^- throughout the growing season via leaf and petiole tissue analysis is a major method of assessing crop nutrient status and demand.

LEAF AND PETIOLE NITROGEN ANALYSIS

Nitrogen (N) is the nutrient that cotton plants respond to most readily. Nitrogen is also considered the most limiting nutrient behind water, thus, N management is one of our most important cotton management tools (Silvertooth et al. 1999). The key to N management is essentially to provide adequate, but not excessive, amounts of plant available N (primarily NO_3^-N) to drive crop development (Olson and Hertz, 1982; McConnell et al., 1996). Improving the elucidation of the present crop N status could alleviate some environmental concerns by decreasing unused N in the soil which could be prone to movement in surface and ground water (McConnell et. al, 2004). Well-established baselines for petiole NO_3^- concentrations in cotton leaf petioles can provide a good method of assessing current crop nitrogen status as the season progresses (Silvertooth et al. 1999). For optimal utilization by the crop, most fertilizer N should be applied between early squaring and peak bloom, in a period of development commonly referred to as "the N application window" (Silvertooth et. al, 1998). The opportunity to correct an N deficiency through a side-dress soil application of N is the most practical, providing the cotton is small enough for ground equipment (Hickey et. al, 1996). The concentration of nitrate-nitrogen in cotton petioles is greatest at pinhead square and gradually decreases as the growing season progresses (Burhan and Babikir, 1968). There is a positive association between the level of nitrogen applied and petiole nitrate-nitrogen, and between petiole nitrate-nitrogen and final yield (Burhan and Babikir, 1968). The greatest predictability of leaf N is observed at peak bloom with a maximum R-square of 0.77 and minimum standard error of prediction of 2.72 (Fridgen and Varco, 2004). Petiole and leaf tissue analysis will vary between vegetative and reproductive growth. Cotton petiole *in vivo* nitrate reductase activity (NRA) is not significantly different from NRA in leaf blades during vegetative growth. On the other hand, *in vivo* NRA in cotton petioles is significantly higher than in leaf blades during reproductive growth (Chu et. al, 1989). Petiole NRA of plants in the vegetative growth stage increased from 39 to 68 pmol $NO_2 g^{-1}$ FW (fresh weight) s^{-1} when supplemented with nitrate-N. This increase in indices that cotton petiole NRA is significant and it has potential as a monitoring system to determine the N concentration in cotton shoots (Chu et. al, 1989).

Nitrogen (N) deficiency limits cotton yields while too much N causes excessive vegetative growth, wastes expensive inputs, and can lead to escape into the environment (Weidenfeld et. al, 2009). Excessive growth also can reduce fiber yield through the routing of assimilates away from reproductive development. Petiole nitrate concentration is found to correlate with cotton N status and is a good indicator of potential growth response to N application (Weidenfeld et. al, 2009). The basis for in-season N fertilizer additions has been NO_3^- monitoring for the most recently matured leaf petiole (Keisling et. al, 1995). Under normal conditions, petiole analysis will indicate N required to maintain adequate growth about 7 to 10 days before the onset of N stress (Hickey et. al, 1996). In previous studies, total leaf N concentrations were found to be less responsive to changes in applied N than were petiole NO_3^- concentrations (Weidenfeld et. al, 2009). The level of petiole NO_3^- showed high positive correlations with the rate of nitrogen application as well as with yield (Burhan and Babikir, 1968). The strong relationship between leaf N and leaf area and boll number ($r^2 = 0.80$ and $r^2 = 0.89$, respectively) one week after first flower appearance suggests that the transition period between vegetative and boll development is a critical time to asses cotton's N status in relation to yield (Gerik et. al, 1994). Petiole and blade nutrient levels were positively correlated amongst P, K, Ca, and Mg (Davis et. al, 1995). Using petiole tissues analysis in this manner, N fertilizer can be adjusted so that N is not limiting during growing seasons and is conducive to high yields (Keisling et. al, 1995).

Yield tends to be maximized with N treatments that included a first square application (McConnel et. al, 2004). Petiole NO_3^- predicted lint yield losses from the week before first bloom until three weeks later with approximately 80% correct estimates. For subsequent weeks of sampling this figure falls to 50% correct estimates (Keisling et. al, 1995). The relationship between leaf N and petiole NO_3^- content for greenhouse grown plants, was best described by $Y = a + bX^C$ where Y is the leaf N content and X is the petiole NO_3^- content.

However, with this function only one-half of the variation in leaf N was accounted for by variation in petiole NO_3^- content (r^2 = .48). Leaf N is a better predictor of cotton vegetative growth (as indicated by LAI) and boll number than petiole NO_3^- content, particularly when measurements are made 1 week after first flower (Gerik, 1994). The shift to bloom starts a general decline in petiole NO_3^- that continues through maturation; In other words as the plant shifts to boll development, less NO_3^- is moved to the terminal for vegetative development because the bolls become stronger sinks than the vegetative portions (Hickey et. al, 1996). Results indicate that petiole NO_3^- content by itself is useful for determining the N nutritional status of cotton until the third week of bloom (Keisling et. al, 1995).

LEAF AND PETIOLE POTASSIUM AND PHOSPHOROUS ANALYSIS

Insufficient potassium (K) nutrition produces detrimental effects on cotton lint yield and fiber quality (Pettigrew and Meredith, 1997). Potassium fertility recommendations based on cotton petiole diagnostic results have been inconsistent in the past, partly because the relative range of acceptable K fertility is unknown and the minimal threshold has not been established (Bednarz et. al, 1999). Petioles are used in weekly nutrient monitoring programs because they are more responsive to short-term changes than leaf blades (Davis 1995). Previous research suggests that seasonal K removal from the soil is approximately 25 kg/ha for cotton in coastal plain soils (Gascho and Parker, 2006). Howard et. al, (1998) reported that foliar applications of K may be used to supplement soil applications of K to maximize yields of cotton. Based on yield, increases from foliar applied K as both buffered solutions and in combination with boron, should return 8 to 10 times the product cost (Howard et. al, 1998). Results from previous research support evidence that K deficiency adversely affects reproductive growth, boll weight, and sugar translocation in cotton (Read et. al, 2006).

Several studies have been performed to determine the correlation of nutrients applied and amount of elemental nutrients found in different sinks throughout the plant. One of these sinks, the leaf blade is the most responsive, whereas the petiole serves more as a nutrient conduit to the reproductive sites (bolls) from the plant proper. One study (Davis1995) examined the influence of time of day and irrigation on petiole and blade phosphorous (P), potassium (K), calcium (C), and magnesium (Mg) concentrations to compare petiole and blade samples in their stability and usefulness in evaluation of cotton nutrient status. The difference in K rates was not as well reflected in the blade K levels as in the petiole K levels (Davis 1995). The onset of K deficiency is first detected in upper canopy petioles and leaves four days after K removal from soil solution (Bednarz et. al, 1996). Previous research also suggests that there are increases of N uptake with sufficient levels of K in the soil (Fridgen and Varco 2004). Potassium further affects boll development along with nitrogen.

Before a N fertilizer management decision is made, the change in petiole phosphorous (P) must also be considered (Hickey et. al, 1996). Estimates of yearly P removal are 11 kg/ha for cotton (Gascho and Parker 2006). An increase in P indicates adequate soil moisture, fruiting is progressing nicely, and, if NO_3^- drops out of the optimum range, a deficiency condition will occur (Hickey et. al, 1996). Phosphorous deficiency results in plants closing stomata when there is significant turgor in the leaf mesophyll (Radin 1984). Sub-optimal levels of phosphorous strongly inhibit leaf expansion in young cotton (Radin and Eidenbock, 1984). Compared to plants grown on high P, plants grown on low P had lower leaf water potentials and transpiration rates, and greater diurnal fluctuations in leaf water potential. In addition, low P significantly decreases root hydraulic conductance and the effects of P nutrition on hydraulic conductance preceded effects on leaf area (Radin and Eidenbock, 1984). Leaves of P-deficient plants accumulated more abscisic acid in response to water stress, but the difference was evident only at low water potentials, after initiation of stomatal closure (Radin, 1984). Data strongly indicate that low P limits leaf expansion by decreasing the hydraulic conductance of the root system (Radin and Eidenbock, 1984).

WATERS INFLUENCE ON NUTRIENT LEVELS IN LEAF AND PETIOLE TISSUE

A specific nutrient deficiency depresses lint yield and fiber development (Read, et. al, 2006). However, water influences cotton growth and development more so than any other factor and is the most limiting factor in any production system (Silvertooth et al. 1999). Nitrogen, potassium, and phosphorous uptake and utilization depend solely on plant available water in the effective rooting zone. Studies performed by Wheeler et. al, (2001) suggest that there is a response to N fertilizer at varying irrigation levels. During years where drought conditions cause water stress and limit plant growth, dry land cotton has a limited response to N fertilizer treatments (McConnel and Mozaffari, 2004). When water is withheld from the pots, stomata of the most recently expanded leaf closed at leaf water potentials of approximately -12 bars (Radin, 1984). Cotton grown in droughty conditions accumulated more abscisic acid in response to water stress and had greater stomatal closure and decreased nutrient movement through the transpiration stream (Radin, 1984). Cotton lint yields showed a quadratic response to irrigation levels (0, 25, 50, or 75% Evapotranspiration replacement) in 1996 and 1997, and a linear response in the drought year of 1998 (Bronson et. al, 2001).

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Using Cotton Leaf and Petiole Tissue to Assess Nitrogen, Phosphorous, and Potassium Interactions within the Cotton Plant

JOHN S. HOLT*, WILLIAM R. FOOTE, GUY D. COLLINS, BRENDA CLEVELAND,

DAVID L. JORDAN, CARL CROZIER, RANDY WELLS and KEITH L. EDMISTEN

J.S. Holt*, K.L. Edmisten, R. Wells, and C. Crozier;

Box 7620, Dept. of Crop Science, North Carolina State University, Raleigh, NC 27695-7620

*Corresponding author email: Seth.Holt@cpsagu.com, phone (919)770-9669

Abbreviations: N, nitrogen; P, phosphorous; K, potassium; NO_3^{-1}, nitrate nitrogen; kg/ha^{-1}, kilograms per hectare; UAN, ammonic, nitrate and urea

ABSTRACT

Tissue sampling has provided a quick and efficient understanding of cotton's current nutritional status throughout the growing season. Several studies have been conducted to determine the optimum amounts of nitrogen (N), phosphorous (P), potassium (K), and nitrate nitrogen ($NO3^{-1}$) within cotton leaf and petiole tissue that will lead to maximum yield and net return through different sampling dates. However, little research has been done in North Carolina to determine the synergistic interaction among N, P, K, and NO_3^{-1} in cotton leaf and petiole tissue. Therefore, the correlation response of N, P, K, and NO_3^{-1} were measured in cotton leaf and petiole tissue based on variable rates of nitrogen and potassium fertilizer on a low K-index soil by the North Carolina Department of Agriculture's soil potassium saturation standard. Treatments consisted of 0, 45, 90, and 134 kg/ha^{-1} nitrogen and 0, 56, and 112 kg/ha^{-1} potassium applied in a randomized block design for a total of 12 treatments. Leaf and petiole tissue were collected once weekly for 8 consecutive weeks beginning at match head square. Data collected from both leaf and petiole tissue samples suggested that lower rates and availability of nitrogen significantly affect the amount of potassium uptake. Higher nitrogen rates (90 and 134 kg ha^{-1}) led to an increase in concentration of leaf and petiole potassium across all experiments. Potassium rates applied in accordance with the North Carolina Department of Agriculture's soil test recommendation, 112 kg/ha^{-1}, resulted in higher correlation values amongst the relationship between petiole nitrogen and potassium.

INTRODUCTION

Monitoring nutrient status in cotton (*Gossypium hirsutum* L.) via tissue sampling has become a common practice among cotton producers across the cotton producing region of the United States. Tissue sampling is performed to examine the current status of specific nutrients in the cotton plant. Tissue testing is a valuable diagnostic tool in identifying nutrient deficiencies that otherwise might be hidden (Stevens et. al, 2002). Plant tissue testing is the most accurate of all diagnostic tools for nutrient deficiencies, particularly when paired samples are taken (Stevens et. al, 2002). Identifying possible nutrient deficiencies can mitigate a reduction in fiber yields (Rochester et. al, 2012). Uptake of nutrients via the roots is governed by nutrient transport to the root surface and adsorbed with the water as part of the transpiration stream (Bassirirad, 2000). Nutrient uptake by cotton is driven by the demand for nutrients from the developing crop, which is regulated by the supply of nutrients from the soil (Rochester et. al, 2012).

Nutrient uptake can be affected by several factors, including nutrient abundance, soil type and soil moisture level. Detecting a nutrient deficiency can be difficult; however, tissue testing is the only way to detect “hidden hunger” in cotton (Stevens et. al, 2002). Being of indeterminate growth habit, cotton is able to take advantages of conditions when they are favorable, so it is tempting to suggest that cotton might be more tolerant to nutrient stress than determinate species and so nutrition management could be more flexible (Rochester et. al, 2012). The rates of nutrient uptake increase at flowering through fruiting, and then slow as the bolls undergo maturation (Mullins and Burmester, 2010). Nitrogen and K are taken up in greatest amounts – at least 200 kg ha^{-1} (Hodges, 1992). Rate of nutrient uptake can be monitored accurately via looking at nutrient concentrations in both leaf and petiole tissue.

Nutrient analysis of plant tissue at different growth stages is a major tool for determining which nutrients are limiting growth (Stevens et. al, 2002). By looking at nutrient accumulation in cotton tissue, specifically cotton leaves, (Thompson et. al, 1976) it has been shown that individual leaf N concentration dropped from 6% to 2% as the leaf aged; with the reduction from day 40 to 60 in leaf age being due to redistribution. Nitrogen, P, and K levels normally decline in leaf tissue as the crop ages as they are either more mobile or marginal in availability and the reductions in leaf N, P, and K may indicate redistribution of nutrients from foliage to developing bolls (Rochester et. al, 2012). The very mobile nature of K, coupled with the ability of many physiological parameters and growth conditions to influence K tissue concentrations, has led to inconsistent reports of critical leaf K values (Kerby and Adams, 1985; Reddy and Zhao, 2005).

While petiole testing for nitrate and potassium concentration has been used for several decades, leaf blade testing is less prone to volatile concentration swings. (Tucker, 1965; Kerby and Adams, 1985; Miley and Maples, 1988; Constable et. al, 1991). Petiole NO_3 and K concentrations decline quickly, making these analyses less useful through the late-flowering and fruiting period than leaf blade testing (Constable et. al, 1991).Petiole testing is more dynamic and nutrient concentrations fluctuate in accordance with prevailing weather conditions, making it less reliable with unfavorable weather conditions (Rochester et. al, 2012). Furthermore, petiole testing has been reported to be too variable and not correlated to yield (Oosterhuis and Morris, 1979). While the smaller decline in tissue nutrient concentrations may make the detection of changes difficult, the concentration of each nutrient can help growers make informed fertilizer management decisions when the idea level is known for each nutrient at that stage of crop growth (Rochester et. al, 2012).

The main objective of this study was to assess the interactions of N, P, and K in cotton leaf and petiole tissue throughout the growth cycle. Secondly, the objective of this study was to assess the true value of leaf versus petiole nutrient concentrations in accordance with variable rates of nitrogen and potassium fertilizer applied.

MATERIALS AND METHODS

Cultural Practices

Field studies were conducted at the Upper Coastal Plain Research Station near Rocky Mount, NC over three years. Cotton was planted with a White 6700 air vacuum 2 row planter in a 91 cm row width with 16 seed m^{-2} on 7 May 2008, 11 May 2009, and 3 May 2010. Plots were 4 rows wide by 15.2 meters long in all three years. Cotton cultivar Deltapine 164 was planted in 2008 and Deltapine 0949 was planted in 2009 and 2010. The experimental design of the trial was a complete randomized block design with four replications in 2008 and 2009 and six replications in 2010.

At the 2008 and 2009 location site, corn was harvested prior to establishing a wheat cover crop in 2007, which was destroyed chemically prior to planting cotton in 2008. A wheat cover crop was established after cotton harvest in 2008 and again chemically destroyed before planting cotton in 2009. At the 2010 location site, corn was harvested prior to establishing a wheat cover crop in 2009, which was chemically destroyed prior to planting cotton in 2010.

All cotton was grown according to recommended agronomic practices published by North Carolina State University and North Carolina Cooperative Extension with the exception of nitrogen and potassium applications. Trial treatments consisted of variable rate 32% nitrogen (urea and ammonium nitrate) applications and variable rate 0-0-60 muriate of potash applications. Nitrogen treatments were 0 g ha, 45 kg ha, 90 kg ha, and 134 kg ha. Potassium treatments were 0 g ha, 56 kg ha, and 112 kg ha. Nitrogen (32% UAN) was broadcast at planting at the rate of 17 kg ha across all plots and environments. Specific potassium treatments were broadcast applied at planting in all three years. Specific nitrogen treatments were banded with a pressurized backpack sprayer at match head square. In 2008 and 2009 both field sites contained a soil Potassium-index of 25 as measured by the North Carolina Department of Agriculture Soil Testing Laboratory. In 2010 the field site contained a soil Potassium-index of 64. The North Carolina Department of Agriculture Soil Testing Laboratory reports soil potassium amounts in indices, with a rating of 25, soil potassium is considered to be low and 64 is considered to be high. In 2008 and 2009 all plots received equal amounts of precipitation from planting to defoliation. In 2010 approximately 3 replications of the trial received consistent precipitation from both rainfall and irrigation, whereas 3 replications received only natural rainfall.

Mepiquat chloride was not applied as a growth regulator in any year. Pre-harvest applications of Ginstar (Thidazuron: N-phenyl-N'-1,2,3-thidiazol-5-urea and Diuron: (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and Def (S,S,S-Tributylphosphorotrithioate). The center two rows were harvested with a John Deere two row spindle picker. Plot seed cotton yield weights were recorded prior to ginning, with a sub-sample of lint weight recorded after ginning. A small table-top saw tooth gin was used for separating cotton lint from seed.

Nutrient Analysis

In all three years, leaf and petiole samples were taken starting at match head square and thereafter every 7 days up through the second week of fruiting (8 weeks). Total leaf nitrogen, phosphorous, and potassium levels were monitored and recorded throughout the 8-week collection period. Petiole nitrogen, phosphorous, potassium, and nitrate levels were also monitored and recorded throughout the 8-week collection period for all three years.

Tissue sampling was performed in accordance with recommendations from the North Carolina Department of Agriculture Plant and Waste Laboratory. Beginning at pinhead square leaf tissue and petiole tissue were collected from the upper most recent mature leaf from 25 randomized plants from the center two rows of every plot. Petioles were immediately separated from the leaf and placed into conjoining containers for tissue drying. Prior to tissue analysis, samples were dried over 96 hours at 80^{o} C then ground with a Wiley Mini-Mill; (Thomas Scientific; Swedesboro, NJ) equipped a 20 mesh (1 mm) screen (Campbell and Plank, 1992). All nitrogen, phosphorous, potassium and nitrate analysis were conducted by the Agronomic Division of the N.C. Department of Agriculture and Consumer Services, Raleigh, NC.

Total nitrogen (N) concentration was determined by NA 1500 oxygen combustion gas chromatography with an elemental analyzer (CE Elantech Instruments; Milan, Italy) (Campbell, 1992). Total concentrations of phosphorus and potassium were determined with an inductively coupled plasma (ICP) spectrometer (Donohue and Aho 1992; USEPA 2001) (Optima 3300 DV ICP emission spectrometer; Perkin Elmer Corporation; Wellesley, MA), after open-vessel HNO_3 digestion in a microwave digestion system (CEM Corp.; Matthews, NC) (Campbell and Plank, 1992). Nitrate nitrogen (NO_3-N) concentration was determined with an ion sensitive electrode (ISE) (Orion Model 93-07; Thermo Fisher Scientific Inc., Waltham, MA) following an $Al_2(SO_4)_3$ extraction (Baker and Smith, 1969).

Economic Return

Economic return was estimated in accordance with North Carolina Cooperative Extension’s Cotton Conventional Tillage budget formula. Fertilizer and chemical applications, fuel for planting and harvesting equipment**,** seed, equipment depreciation, and labor were all input costs accounted for in determining net profit. Nitrogen fertilizer costs were $1.01/kg in 2008, $1.63/kg in 2009, and $0.73/kg in 2010. Potassium fertilizer costs were $0.51/kg in 2008 and 2009, and $1.18/kg in 2010. Average selling price of cotton was $1.65/kg in 2008, $1.61/kg in 2009, and $1.72/kg in 2010.

RESULTS

The influence of year, nitrogen fertilization, and potassium fertilization were measured by lint yield and net return (Table 1). Nitrogen rate and K rate both had significant influence on both yield and net return across all experiments. The interaction among N and K rates resulted in both positive and negative net returns across treatments in all years and were dependent on fertilizer rate and yield (Table 1). The interaction of year by N rate and K rate reveals that the highest yields were achieved with application rates of 134 kg/ha^{-1} nitrogen and 112 kg/ha^{-1} potassium in 2008, 134 kg/ha^{-1} nitrogen and 56 kg/ha^{-1} potassium in 2009, and 90 kg/ha^{-1} nitrogen and 56 kg/ha^{-1} in 2010. Mean yields for 2008, 2009, and 2010 were 885, 682, and 617, respectively.

Nutrient concentration (N and K) in petiole tissue was significantly influenced by application rates as indicated by the Pearson correlations for nitrogen and potassium concentrations (Table 2). In both 2009 and 2010, petiole potassium concentrations tended to increase with applied potassium fertilizer. Higher correlations of petiole nitrogen and potassium were observed when higher levels of potassium fertilizer were applied.

Tissue nutrient measurements were taken once per week beginning at match head square and nutrient interaction within the leaf and petiole tissue was observed. Year, nitrogen rate, and year x nitrogen rate were significant within leaf tissue when measuring leaf nitrogen concentration across all experiments (Table 3). Year was significant at all measurement times. Nitrogen, Year x N, and Year x K were significant for 3 weeks and later. Potassium rate was not significant for any week of measurement. For the petioles, year was significant for all weeks except weeks 4 and 7. Nitrogen and K rates were significant for weeks 4 and later. Year x N rate was significant for weeks 4 and later and Year x K rate was significant for weeks 5, 6, and 8.

Potassium concentration in leaf tissue was significantly affected by year, for weeks 2 and later (Table 5). The effect of nitrogen rate on potassium was significant for weeks 2, 6, 7, and 8. Potassium rate was significant for weeks 6 and later. Petiole nitrogen concentration was affected in weeks 5, 6, and 8 following match head square in 2009 and 2010 in response to all three rates of potassium fertilizer (Table 6). Potassium concentration was significant for year and most weeks whether it be leaf or petiole (Table 5). The same was true for the effect of K rate with a significant interaction for all weeks in leaf and petiole. Nitrogen rate was significant in leaves for weeks 4, 5, and 8 and in petioles for weeks 5 and later. Petiole potassium levels responded significantly to four different levels of nitrogen fertilization in weeks 5, 6, and 8 following match head square in 2009 and 2010 (Table 7).

Leaf potassium concentration was highest when potassium fertilizer was applied at112 kg/ha^{-1} across all experiments (Figure 1). The highest potassium concentration in leaf tissue was achieved when potassium fertilizer was applied at 112 kg/ha^{-1} and nitrogen fertilizer was applied at 45, 90, and 134 kg/ha^{-1}. Petiole nitrogen levels were consistently higher when higher rates of nitrogen fertilizer were applied (Figure 2). The highest nitrogen concentrations in petiole tissue were achieved when nitrogen fertilizer was applied 134 kg/ha^{-1} and potassium fertilizer was applied at 0 and 56 kg/ha^{-1}. Petiole potassium levels were all higher when potassium fertilizer was applied at 112 kg/ha^{-1} and nitrogen fertilizer was applied at 45 kg/ha^{-1} and higher (90 kg/ha^{-1} and 134 kg/ha^{-1}) (Figure 3). Petiole nitrate concentrations were all high when nitrogen fertilizer was applied at the highest rate of 134 kg/ha^{-1} (Figure 4). Potassium fertilizer had no significant influence on petiole nitrate concentrations across all experiments.

In all years and experiments, soil moisture influence on nutrient uptake and nutrient status within leaf and petiole tissue was extremely significant. Both nitrogen and potassium levels in petiole tissue consistently followed rain events across all sampling dates. When nitrogen and potassium fertilizer were applied at 0 kg/ha^{-1}, nitrogen and potassium levels in petiole tissue began at a high concentration at match head square, spiked with rainfall, and continued to decrease as the growing season progressed(Figure 5). Similar results were obtained when nitrogen fertilizer was applied at 45 kg/ha^{-1} and potassium fertilizer was applied at 56 kg/ha^{-1} (Figure 6). There was a significant difference in the longevity of both nitrogen and potassium levels in petiole tissue when more nitrogen and potassium fertilizer was applied (Figure 6) versus no fertilizer application (Figure 5). When nitrogen and potassium fertilizer were applied at the highest rates (134 kg/ha^{-1} nitrogen and 112 kg/ha^{-1} potassium), petiole nitrogen and potassium levels reached their highest levels with every rainfall event, and maintained a higher nutrient status throughout the remaining sampling dates (Figure 7). Potassium levels in petiole tissue were able to maintain at a higher concentration when potassium fertilizer was applied at 112 kg/ha^{-1} (Figure 8). However, petiole potassium levels did not reach as high of a concentration when nitrogen was completely lacking throughout all sampling dates (Figure 8.) versus when the maximum rate of nitrogen fertilizer was applied (Figure 7). Petiole nitrogen concentrations steadily responded to rainfall and higher rates of nitrogen fertilization (134 kg/ha^{-1}) with the highest petiole nitrogen concentration reaching 4% the fourth week following match head square in 2009 (Figure 9).

DISCUSSION

Nitrogen and potassium fertilizer rates had significant influence on both yield and net return in all years and locations. Yield and economic return as affected by nitrogen and potassium fertilization over three years coincided with previous research suggesting nutrient stress in Upland cotton depresses lint yield, particularly of late-season fruit and may disrupt fiber development (Read, et. al, 2006). Cotton however might be more tolerant to nutrient stress than determinate species and so nutrition management could be more flexible (Rochester et. al, 2012). Lint yields were greatest when the highest rate of nitrogen and potassium were applied in 2008, and higher rates of nitrogen and potassium (134 kg/ha^{-1} nitrogen and 56 kg/ha^{-1} potassium) in 2009. In 2010, the lack of yield response between the two higher treatments of nitrogen may be due to ample amounts of soil mineral nitrogen (Gascho et. al, 2006) that was residing from the previous year's corn crop. Due to drought in 2009, the amount of residual soil nitrogen from the previous corn crop could have caused the lack of response seen between petiole nitrogen and potassium in 2010. Correct nitrogen rates to meet crop demand play a major role in lint production (Hickey et. al, 1996). Observations made during the 2008, 2009, and 2010 growing season were supported by previous research suggesting nitrogen deficiency decreased yield through early termination of reproductive growth (Read et. al, 2006).

Spikes in both nitrogen and potassium levels seemed to follow precipitation more so than physiological development, thus making it difficult to monitor plant development with previous research suggesting that during square ontogeny, nitrogen concentrations of sympodial leaves and floral bracts decrease (Zhao et. al, 1999). Also, the rate of nutrient uptake increases at flowering through fruiting, and then slows as the bolls mature (Mullins and Burmester, 2010). Data collected supported that the uptake of nutrients via the roots is governed by nutrient transport to the root surface and absorbed with the water as part of the transpiration stream; or become concentrated in the xylem sap due to a facilitated (protein transporter) or an active uptake process that requires metabolic energy to overcome a concentration gradient (Bassirirad, 2000). The fluctuation in precipitation also did not allow the observation during square development, the effect of main-stem node and sympodial branch fruiting position in the plant canopy on mineral nutrient element concentrations of bracts are greater than those on floral buds (Zhao et. al, 1999). Also, nitrogen accumulation in cotton bolls occurs relatively early, unlike other nutirents that accumulate as the boll matures (Rochester et. al, 2012.) Nitrogen's fast mobility throughout the plant, with cotton seed accumulating 3.5% of total nitrogen fertilizer applied (Egelkraut et. al, 2004). Therefore, nitrogen's fast mobility can pose difficulty in monitoring complete nitrogen uptake throughout the growing season. Yield results in 2008 and 2009 proved that insufficient potassium nutrition produces detrimental effects on cotton lint yield and quality (Pettigrew, et. al, 1997). In 2008 profit margins were higher when the combination of nitrogen was applied at 90 kg/ha^{-1} or greater and potassium was applied at 112 kg/ha^{-1}

proving profit margins are expanded when the uptake efficiency of nitrogen is increased (McConel et. al, 2004).

Nitrogen and potassium levels in petiole tissue were consistently higher following precipitation. Furthermore, both nitrogen and potassium levels maintained a higher nutritional status within petiole tissue throughout the growing season when higher rates of nitrogen and potassium fertilizer were applied. Petiole tissue provided a more accurate analysis of plant nutrition in regards to sampling time. (Thompson et. al, 1976) showed that individual leaf nitrogen concentration dropped from 6% to 2% as the leaf aged; with the reduction from day 40 to 60 in leaf age being due to redistribution. For a typical leaf the amount exported would be about 50% of leaf nitrogen (Zhu and Oosterhuis, 1992). In addition, nitrogen and phosphorous levels normally decline in leaf tissue as the crop ages due to those nutrients being more mobile marginal in availability (Rochester et. al, 2012). There is a good agreement between boll data and crop data in that larger proportions of nitrogen taken up by the crop into vegetative material are redistributed to the developing bolls and removed at harvest (Rochester, 2007) with only 17% of total plant boll nitrogen be supplied by redistribution of leaves alone (Kerby et. al, 1987; Constable, 1991). Petiole testing is more dynamic and nutrient concentrations fluctuate in accordance with prevailing weather conditions (Oosterhuis and Morris, 1979.) The decline in nitrogen content in leaves, stems, petioles, boll walls, bracts, and lint may be equated to 2/3 the nitrogen found in the seed at maturity (Errington et. al, 2009). The very mobile nature of potassium, coupled with the ability of many physiological parameters and growth conditions to influence potassium tissue concentrations, has led to inconsistent reports of critical leaf potassium values (Kerby and Adams, 1985; Reddy and Zhao, 2005).

Cotton is reputed to be less efficient for potassium uptake from soil than other crops (Cope 1981). As a result of potassium being less mobile than nitrogen, under less favorable weather conditions in all years, lower potassium levels in petiole tissue resulted in lower yields. The relationship between applied nitrogen and potassium fertilizer resulted in a higher correlation of nitrogen and potassium concentration in petiole tissue when higher levels of potassium fertilizer were applied. This correlation proves that following the soil test report suggesting that an application of 112 kg/ha potassium be applied on a low K index soil will result in both higher potassium uptake and yield. However, applying potassium on a high K index soil, as in 2010, resulted in lower correlation values between petiole nitrogen and potassium. Lower yields from lack of proper potassium fertilization were a result of deficiency in potassium to maintain fiber cell turgor pressure and so facilitating fiber elongation (Rochester et. al, 2012). (Pettigrew 1996) reported reductions in fiber elongation and other fiber quality properties in response to potassium deficiency. Results from 2009 and 2010 coincided with previous research proving that the relationship between fertilizer N requirement and petiole nitrate concentration is exponential, with greatest precision when high fertilizer rates were required. The relationship between fertilizer N requirement and the rate of decline in petiole nitrate was linear, giving equal precision over the N fertilizer range (Constable et. al, 1991).

Petiole phosphorous levels were never significantly affected by both nitrogen and potassium fertilization treatments. Due to a high level of soil phosphorous, this could have inhibited a response of petiole phosphorous from different fertilizations treatments of nitrogen and potassium. Had the soil been deficient in phosphorous, we could have seen a greater reaction between nitrogen concentration as previous research suggests crop response to N is greatly reduced when P is limiting (Havlin et. al, 2005). Leaf phosphorous levels, however, were highest when nitrogen fertilizer treatments were lowest (0 and 45 kg/ha^{-1}) and potassium fertilizer was applied at 56 kg/ha^{-1}. Petiole phosphorous concentrations never responded to rainfall events, in fact petiole phosphorous levels flat lined across all sampling dates and experiments.

CONCLUSION

Correlations between nitrogen and potassium were consistent with previous research, in that nitrogen and potassium concentrations were affected by fertilizer rate, rainfall, and nutrient interaction within the plant. Petiole nitrogen concentration was directly affected across all experiments by amount of nitrogen fertilizer applied and precipitation. Greater nitrogen fertilizer applications resulted in larger petiole nitrogen concentrations, especially after rainfall events. Greater nitrogen fertilizer applications also resulted in petiole tissue maintaining a higher level of nitrogen throughout the growing season. In most years, higher nitrogen rates resulted in higher yields and net return. The observed higher nitrogen concentration within petiole tissue was not directly influenced by potassium fertilizer rate.

Potassium concentration within petioles behaved similarly to nitrogen concentration, in that it responded to potassium application rate and rainfall. Potassium also remained at a higher status within petiole tissue throughout the growing season when greater rates of potassium fertilizer were applied. However, potassium concentration within petiole tissue was affected by nitrogen fertilizer rate in some years, with petiole potassium levels increasing with an increase in nitrogen application. The interaction between nitrogen rate and potassium concentration, measured by petiole potassium concentration indicates that potassium uptake is limited by available nitrogen to the cotton plant during the growing season. Also, there is a higher correlation between petiole nitrogen and potassium concentrations when higher levels of potassium were applied on a low K index soil. When potassium fertilizer was applied in accordance with the North Carolina Department of

Agriculture’s soil test recommendation, higher petiole nitrogen and potassium correlations were observed. Using this interaction between nitrogen and potassium fertility rates, concentration of petiole potassium, and rainfall makes fertility adjustments based on petiole potassium concentration seemingly plausible.

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Table 1. Yield and net economic return as affected by nitrogen and potassium fertilization over three years.

		Lint Yield			Net Return		
Nitrogen	Potassium	2008	2009	2010	2008	2009	2010
g/ha^{-1}	kg/ha^{-1}	kg/ha^{-1}			\$/ha^{-1}		
0	0	630.92	559.02	611.03	87.06	-166.14	91.70
0	56	753.13	620.98	657.64	260.14	-89.94	105.79
0	112	812.81	640.59	620.98	330.05	-86.93	-23.35
45	0	844.07	681.37	690.61	393.30	-37.51	197.07
45	56	831.99	726.70	618.42	344.81	6.91	6.83
45	112	960.60	727.70	623.53	528.46	-20.04	-50.46
90	0	789.37	741.34	602.22	257.60	-14.31	13.54
90	56	1009.63	650.16	662.19	595.16	-189.66	50.61
90	112	1091.33	703.54	603.93	698.71	-132.29	-115.68
134	0	875.34	566.13	541.69	355.01	-368.11	-121.36
134	56	920.09	790.36	590.31	400.28	-35.67	-103.82
134	112	1095.59	773.45	576.36	661.30	-34.84	-193.90
LSD		45.61	29.27	30.64	57.09	36.63	38.78

†Net Return was calculated using North Carolina Cooperative Extension's Cotton Conventional Tillage budget formula.
‡Nitrogen fertilizer costs were \$1.01/kg (2008), \$1.63/kg (2009), and \$0.70/kg (2010)
§Potassium fertilizer costs were \$0.51/kg (2008 & 2009) and \$1.18/kg (2010)
¶ Average selling price was \$1.65/kg (2008), \$1.61/kg (2009), and \$1.72/kg (2010)

Table 2. Pearson correlations for nitrogen and potassium concentrations in petiole tissue over all sampling dates as affected by nitrogen and potassium fertilization over two years.

Nitrogen	Potassium	Petiole N and K	
		2009	2010
kg/ha^{-1}	kg/ha^{-1}	------------------R-----------------	
0	0	0.57*	0.03
0	56	0.63*	0.08
0	112	0.82*	0.27
45	0	0.56*	0.02
45	56	0.68*	0.29
45	112	0.85*	0.35
90	0	0.51*	0.22
90	56	0.83*	0.07
90	112	0.68*	0.08
134	0	0.32	0.32
134	56	0.59*	0.24
134	112	0.60*	0.64*

†Nitrogen fertilizer treatments were applied banding 30% liquid UAN at pin head square.
‡Potassium fertilizer treatments were applied broadcasting Muriate of Potash at planting.
§* Indicates significant values for variables at $P \leq 0.050$.

Table 3. Analysis of variance for nitrogen concentration in cotton leaves and petioles in response to nitrogen, year, potassium, and their interaction for various weeks starting at match head square.

Source	Weeks starting at match head square†							
	1†	2	3	4	5	6	7	8
				Leaf				
Year	<0.0001	0.0001	<0.0001	<0.0001	0.0024	<0.0001	<0.0001	<0.0001
Nitrogen rate (N rate)‡	0.5380	0.4196	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Potassium rate (K rate)§	0.0840	0.2360	0.8120	0.5182	0.7627	0.6342	0.5619	0.7098
Year * N rate	0.9593	0.7747	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year * K rate	0.9762	0.2130	0.9532	0.6598	0.3335	0.9681	0.5311	0.5693
N rate * K rate	0.7946	0.7099	0.6422	0.2439	0.3143	0.7644	0.0915	0.7530
Year * N rate * K rate¶	0.9075	0.8030	0.8414	0.9957	0.7132	0.6018	0.7095	0.6488
Coefficient of variation (%)	7.40	7.26	8.46	7.11	9.50	6.72	6.27	6.75
No. of years#	3	3	3	3	3	3	3	3
				Petiole				
Year	-	0.0057	0.0435	0.2592	0.0005	0.0020	0.9734	0.0007
Nitrogen rate (N rate)	-	0.8741	0.2737	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Potassium rate (K rate)	-	0.7063	0.5624	0.0527	0.0013	<0.0001	0.0158	0.0006
Year * N rate	-	0.7706	0.5566	0.9616	0.0016	<0.0001	0.0184	<0.0001
Year * K rate	-	0.5321	0.1986	0.1855	0.0011	0.0018	0.9673	0.0384
N rate * K rate	-	0.3730	0.1395	0.7490	0.5800	0.6821	0.7545	0.9829
Year * N rate * K rate	-	0.3990	0.7745	0.7844	0.4913	0.8034	0.9037	0.7286
Coefficient of variation (%)	-	16.96	17.40	16.33	25.03	13.39	18.87	17.39
No. of years#	-	2	2	2	2	2	2	2

† Match head square starts at week 1.
‡ N rate combines all nitrogen (N) fertilizer treatments over all experiments.
§ K rate combines all potassium (K) fertilizer treatments over all experiments.
¶ Year * N rate * K rate measures the interaction among nitrogen and potassium fertilizer treatments over all experiments.
# Number of years is 2 for 2009 and 2010 whereas 3 includes 2008.

Table 4. Analysis of variance for phosphorous concentration in cotton leaves and petioles in response to year, nitrogen, potassium, and their interaction at various dates starting at match head square.

	Weeks starting at match head square†							
Source	1	2	3	4	5	6	7	8
				Leaf				
Year	0.5586	<0.0001	<0.0001	<0.0001	0.8084	<0.0001	<0.0001	0.0002
Nitrogen rate (N rate)‡	0.5170	0.0520	0.5480	0.1161	0.3914	0.0046	0.0004	0.0262
Potassium rate (K rate)§	0.1603	0.6540	0.8410	0.3245	0.3864	0.0020	0.2299	0.2101
Year * N rate	0.6906	0.2825	0.5380	0.0467	0.4192	0.0016	0.0002	0.0006
Year * K rate	0.1011	0.1855	0.3343	0.0142	0.4038	0.3979	0.1535	0.7882
N rate * K rate	0.4836	0.4170	0.7011	0.2224	0.4177	0.3621	0.7528	0.7054
Year * N rate * K rate¶	0.5786	0.9173	0.0770	0.8674	0.4762	0.6216	0.1924	0.8799
Coefficient of variation (%)	54.15	10.05	9.78	9.89	180.99	11.07	13.59	12.47
No. of years#	3	3	3	3	3	3	3	3
				Petiole				
Year	-	0.1940	0.0039	0.0077	0.0003	0.0003	<0.0001	0.0003
Nitrogen rate (N rate)	-	0.6270	0.4924	0.5993	0.6212	0.0055	0.1958	0.0192
Potassium rate (K rate)	-	0.0191	0.0004	0.0028	0.0277	0.1720	0.3565	0.1159
Year * N rate	-	0.8225	0.5584	0.6551	0.5158	0.0040	0.0375	0.0260
Year * K rate	-	0.9932	0.0228	0.4934	0.1135	0.3320	0.0487	0.5438
N rate * K rate	-	0.3211	0.4673	0.6470	0.9440	0.9220	0.6937	0.2122
Year * N rate * K rate	-	0.0193	0.7696	0.8980	0.8821	0.3907	0.6363	0.3042
Coefficient of variation (%)	-	14.37	13.77	16.73	22.33	15.98	17.22	15.34
No. of years	-	2	2	2	2	2	2	2

† Match head square starts at week 1.
‡ N rate combines all nitrogen (N) fertilizer treatments over all experiments.
§ K rate combines all potassium (K) fertilizer treatments over all experiments.
¶ Year * N rate * K rate measures the interaction among nitrogen and potassium fertilizer treatments over all experiments.
# Number of years is 2 for 2009 and 2010 whereas 3 includes 2008.

Table 5. Analysis of variance for potassium concentration in cotton leaves and petioles in response to year, nitrogen, potassium, and their interaction at various dates starting at match head square.

	Weeks starting at match head square†							
Source	1	2	3	4	5	6	7	8
				Leaf				
Year	<0.0001	<0.0001	0.0021	0.0071	0.0054	0.0098	0.0924	0.0016
Nitrogen rate (N rate)‡	0.3828	0.6938	0.2787	0.0025	0.0029	0.5422	0.7240	0.0476
Potassium rate (K rate)§	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year * N rate	0.7098	0.4507	0.7732	0.7875	0.2387	0.6763	0.1380	0.2336
Year * K rate	0.4431	0.0708	0.6601	0.0051	0.4731	0.3095	0.0417	0.0798
N rate * K rate	0.4246	0.8597	0.8379	0.3168	0.2311	0.2990	0.8429	0.6835
Year * N rate * K rate¶	0.6786	0.1814	0.1735	0.8640	0.8979	0.4255	0.7376	0.6101
Coefficient of variation (%)	19.17	16.53	17.30	15.17	15.47	20.01	18.51	15.71
No. of years#	3	3	3	3	3	3	3	3
				Petiole				
Year	-	<0.0001	0.0001	0.0001	0.0854	0.0465	0.0059	0.1008
Nitrogen rate (N rate)	-	0.6948	0.5023	0.3115	0.0002	<0.0001	0.0039	<0.0001
Potassium rate (K rate)	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year * N rate	-	0.4347	0.7280	0.5703	0.0035	0.0048	0.1012	0.0070
Year * K rate	-	0.2013	0.5729	0.0687	0.0932	0.0060	0.0001	0.0077
N rate * K rate	-	0.6072	0.2454	0.1847	0.7639	0.1937	0.1096	0.0765
Year * N rate * K rate	-	0.8246	0.9581	0.9745	0.6779	0.6608	0.6065	0.2447
Coefficient of variation (%)	-	10.26	13.22	18.54	14.32	16.27	16.83	18.12
No. of years	-	2	2	2	2	2	2	2

† Match head square starts at week 1.
‡ N rate combines all nitrogen (N) fertilizer treatments over all experiments.
§ K rate combines all potassium (K) fertilizer treatments over all experiments.
¶ Year * N rate * K rate measures the interaction among nitrogen and potassium fertilizer treatments over all experiments.
# Number of years is 2 for 2009 and 2010 whereas 3 includes 2.

Table 6. Response of petiole nitrogen concentration at various weeks starting at match head square to three levels of potassium fertilization.

Potassium	N Concentration		
	Weeks starting at match head square		
	5	6	8
kg/ha-1	-----------------------------	%	-----------------------------
		2009	
0	2.97	2.45	2.41
56	2.57	2.04	2.06
112	2.02	1.95	1.90
		2010	
0	1.51	1.73	1.59
56	1.40	1.62	1.48
112	1.51	1.70	1.48
LSD (0.05)	0.35	0.18	0.22

†Match head square starts at week 1.

‡Weeks 5, 6, and 8 following match head square represented the most significant response of petiole nitrogen concentration to potassium fertilization.

§Petiole data was collected in 2009 and 2010.

Table 7. Response of petiole potassium concentration at various weeks starting at match head square to four levels of nitrogen fertilization.

Nitrogen	K Concentration		
	Weeks starting at match head square		
	5	6	8
kg/ha-1	----------------------------%----------------------------		
		2009	
0	2.58	1.87	1.86
45	3.08	2.40	2.77
90	3.49	2.82	3.01
134	3.68	3.10	3.31
		2010	
0	3.40	2.98	2.98
45	3.58	3.25	3.19
90	3.50	3.24	3.23
134	3.55	3.29	3.39
LSD (0.05)	0.39	0.38	0.44

†Match head square starts at week 1.

‡Weeks 5, 6, and 8 represented the most significant response of petiole potassium concentration to nitrogen fertilization.

§Petiole data was collected in 2009 and 2010.

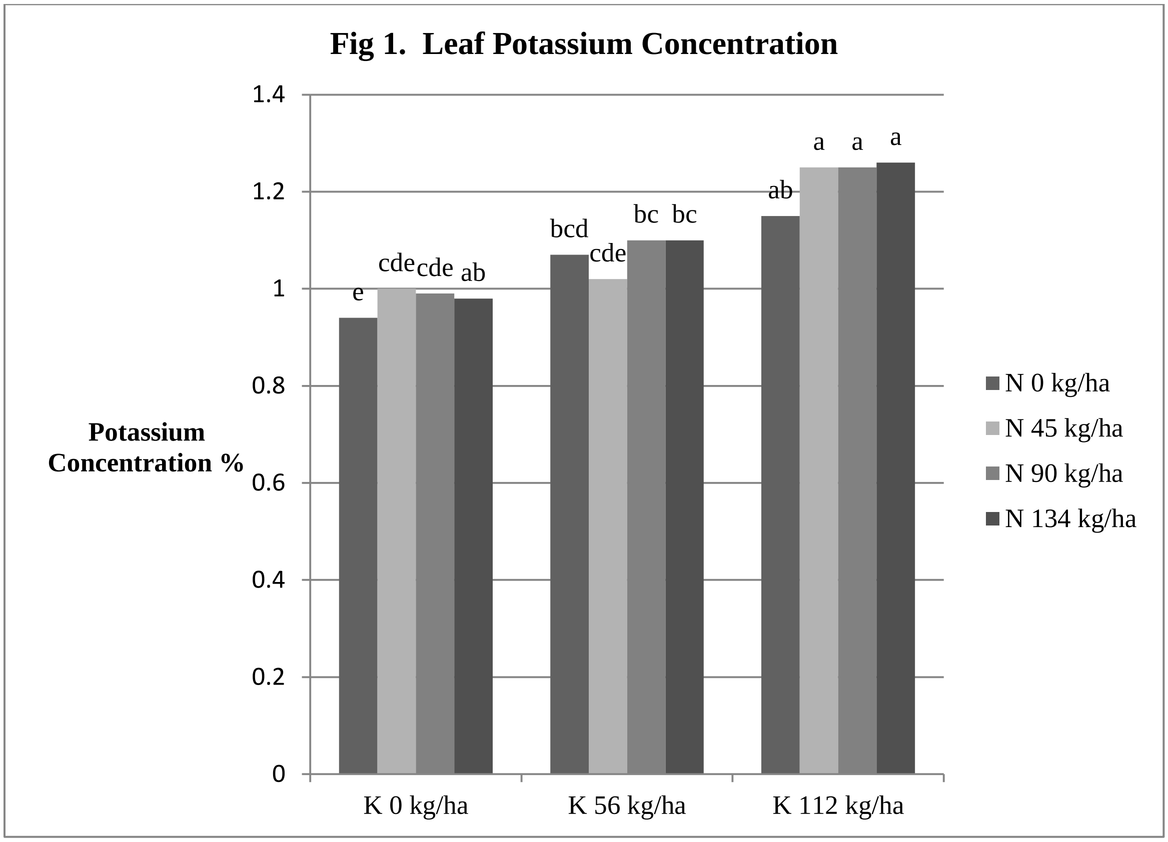

Figure 1. Response of leaf potassium concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.

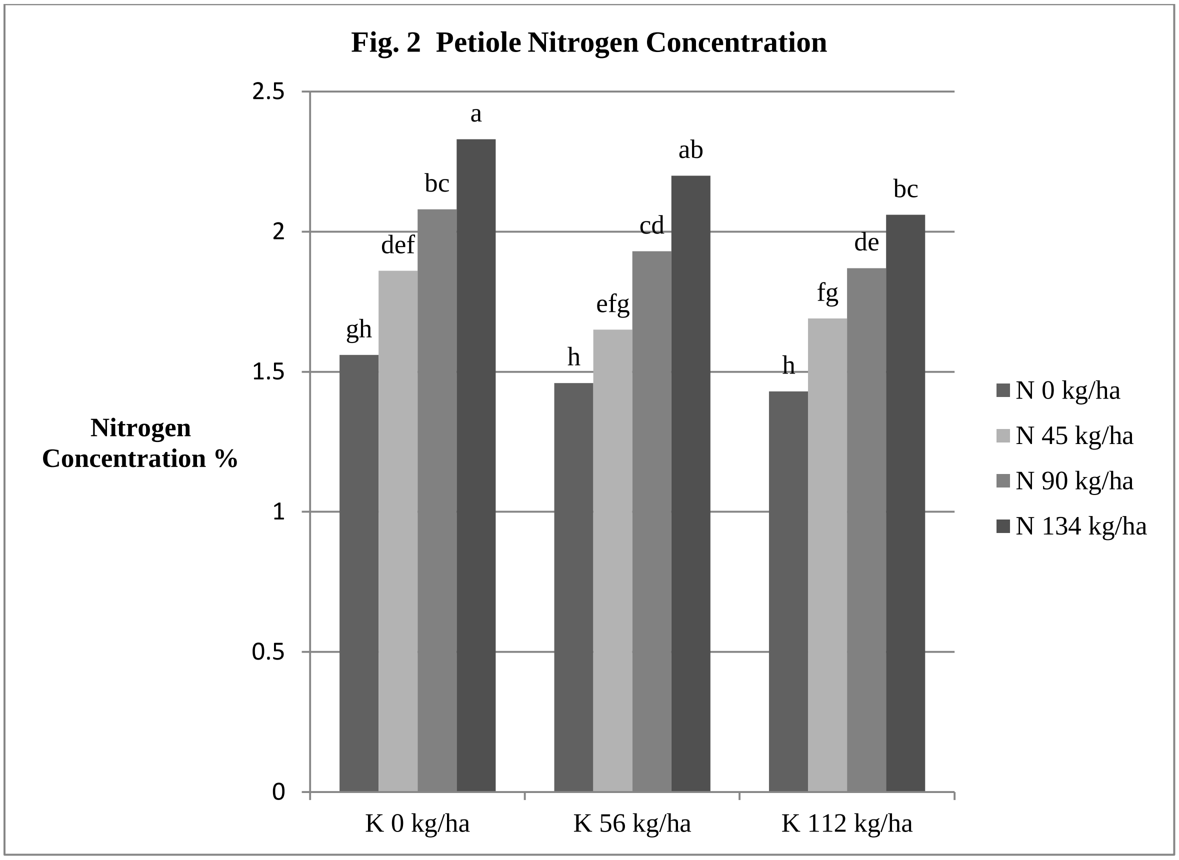

Figure 2. Response of petiole nitrogen concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.

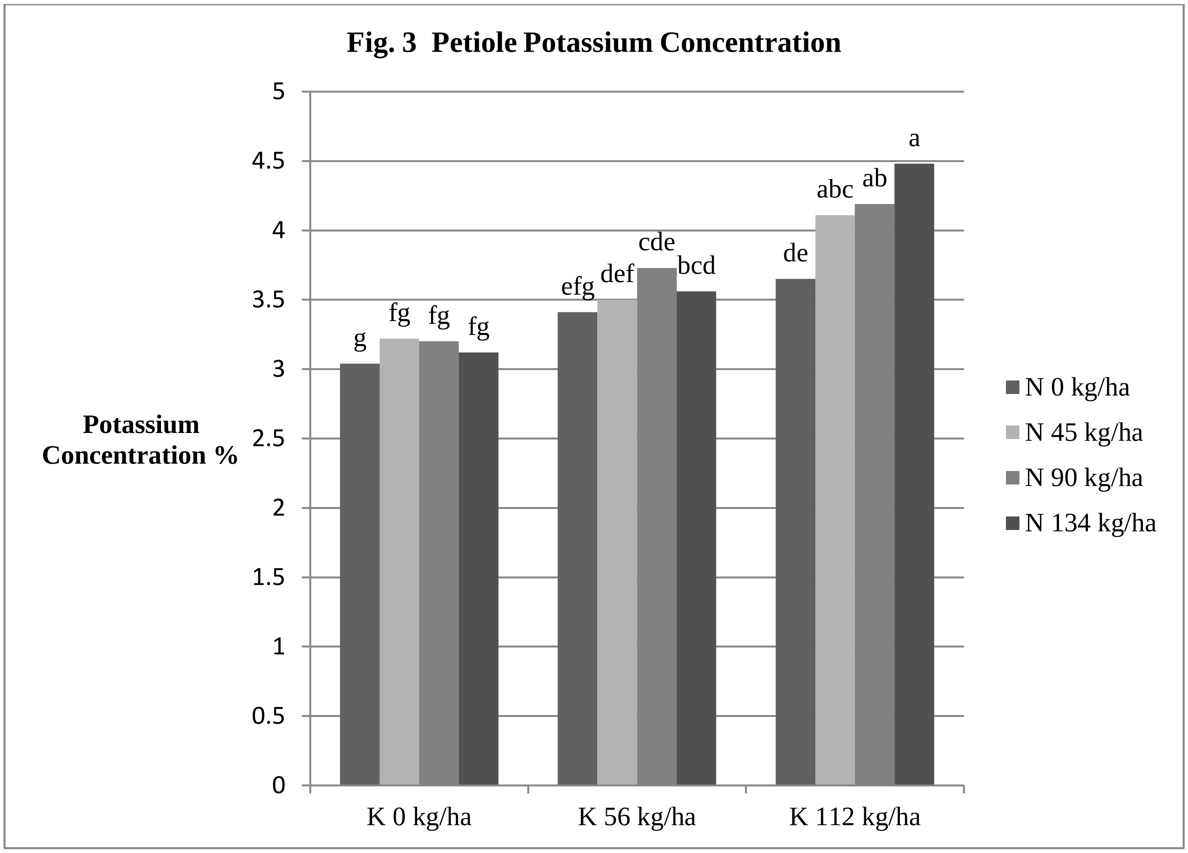

Figure 3. Response of petiole potassium concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.

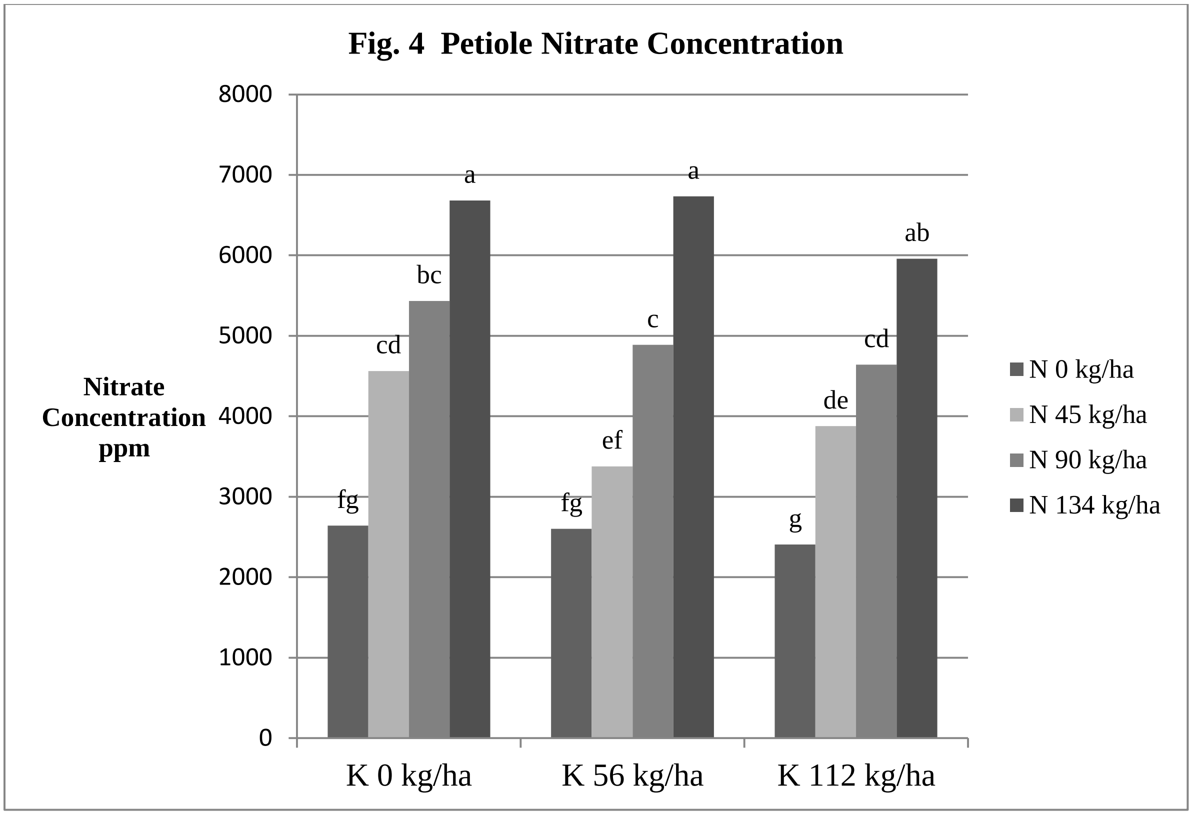

Figure 4. Response of petiole nitrate concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.

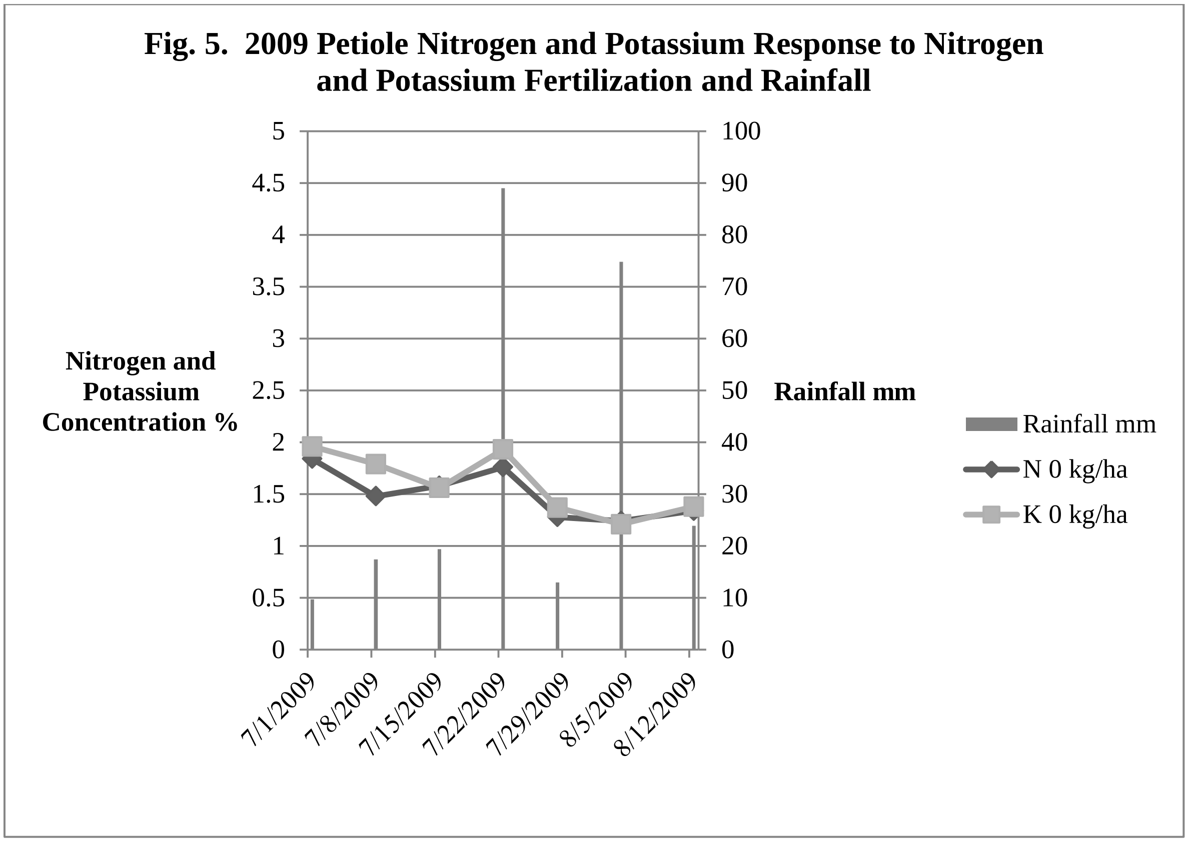

Figure 5. Response of petiole nitrogen and potassium concentration by sampling date beginning at match head square to one level of nitrogen (N) and potassium (K) fertilization at the Upper Coastal Plain Research Station in 2009.

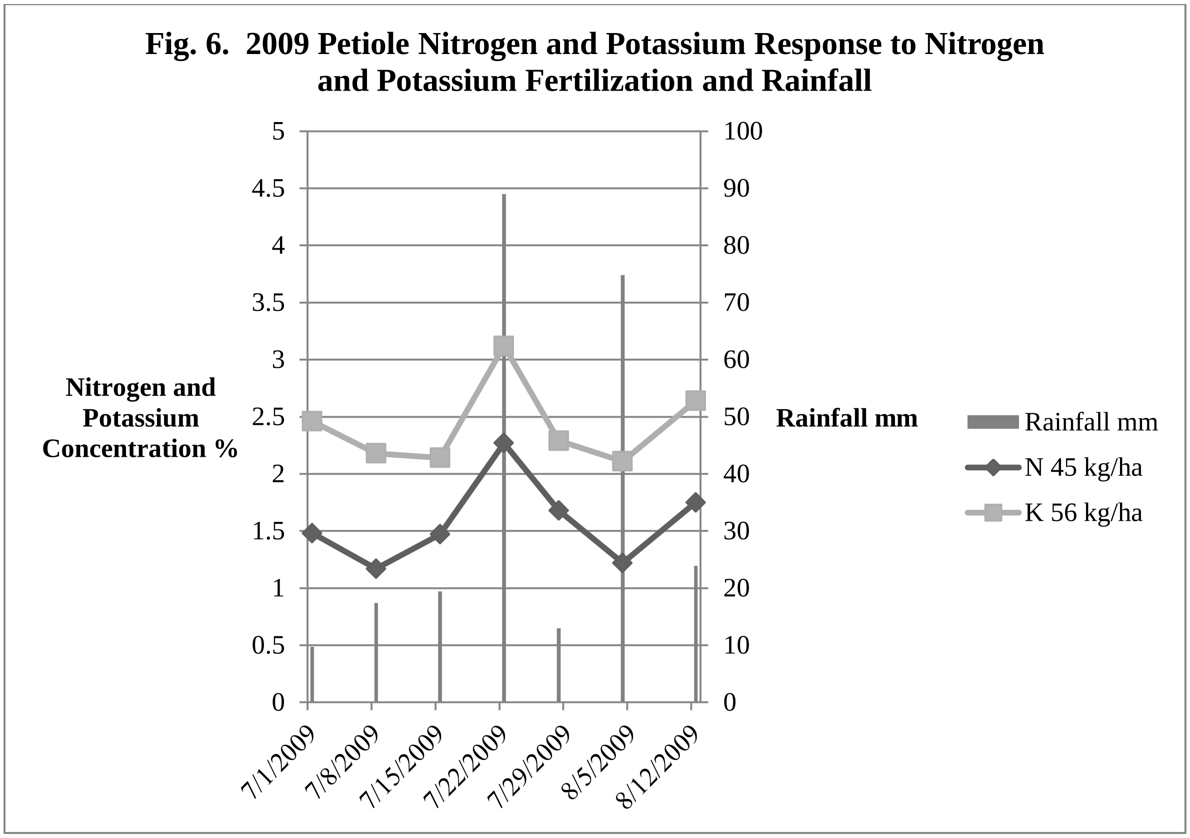

Figure 6. Response of petiole nitrogen and potassium concentration by sampling date beginning at match head square to one level of nitrogen (N) and potassium (K) fertilization at the Upper Coastal Plain Research Station in 2009.

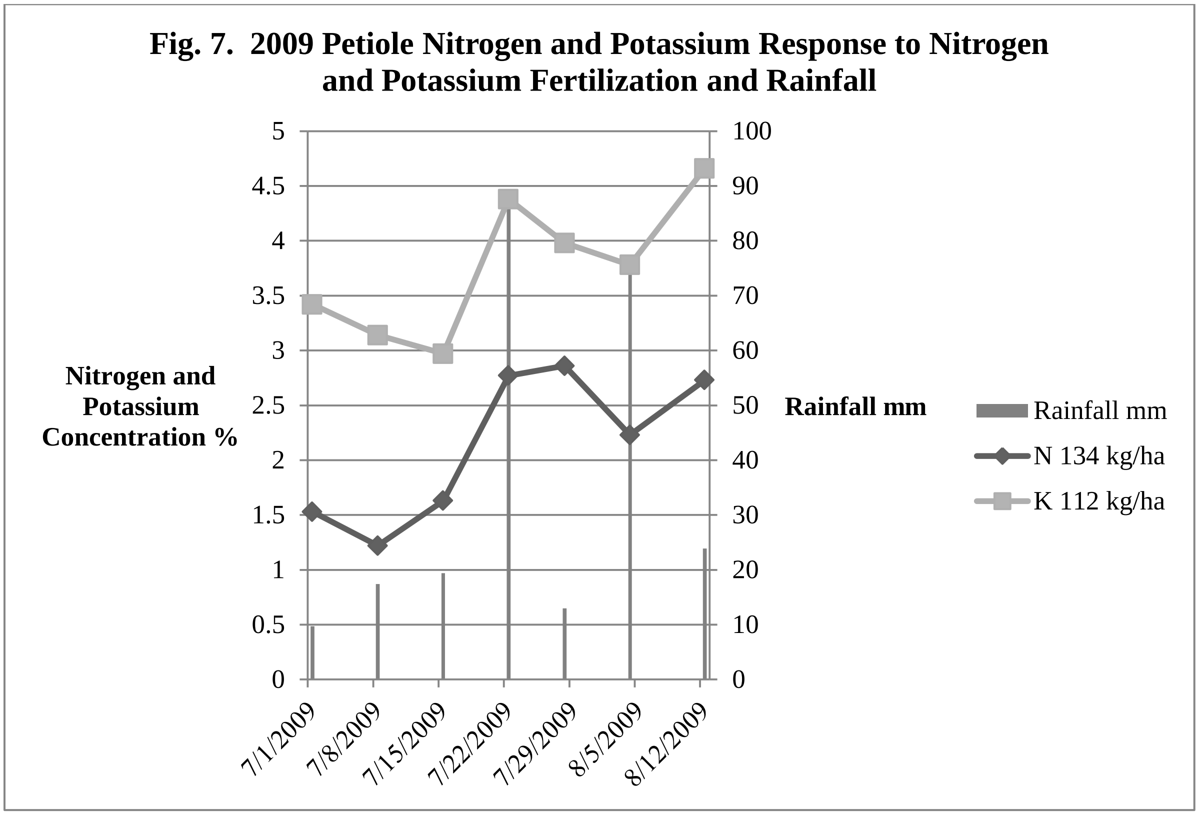

Figure 7. Response of petiole nitrogen and potassium concentration by sampling date beginning at match head square to one level of nitrogen (N) and potassium (K) fertilization at the Upper Coastal Plain Research Station in 2009.

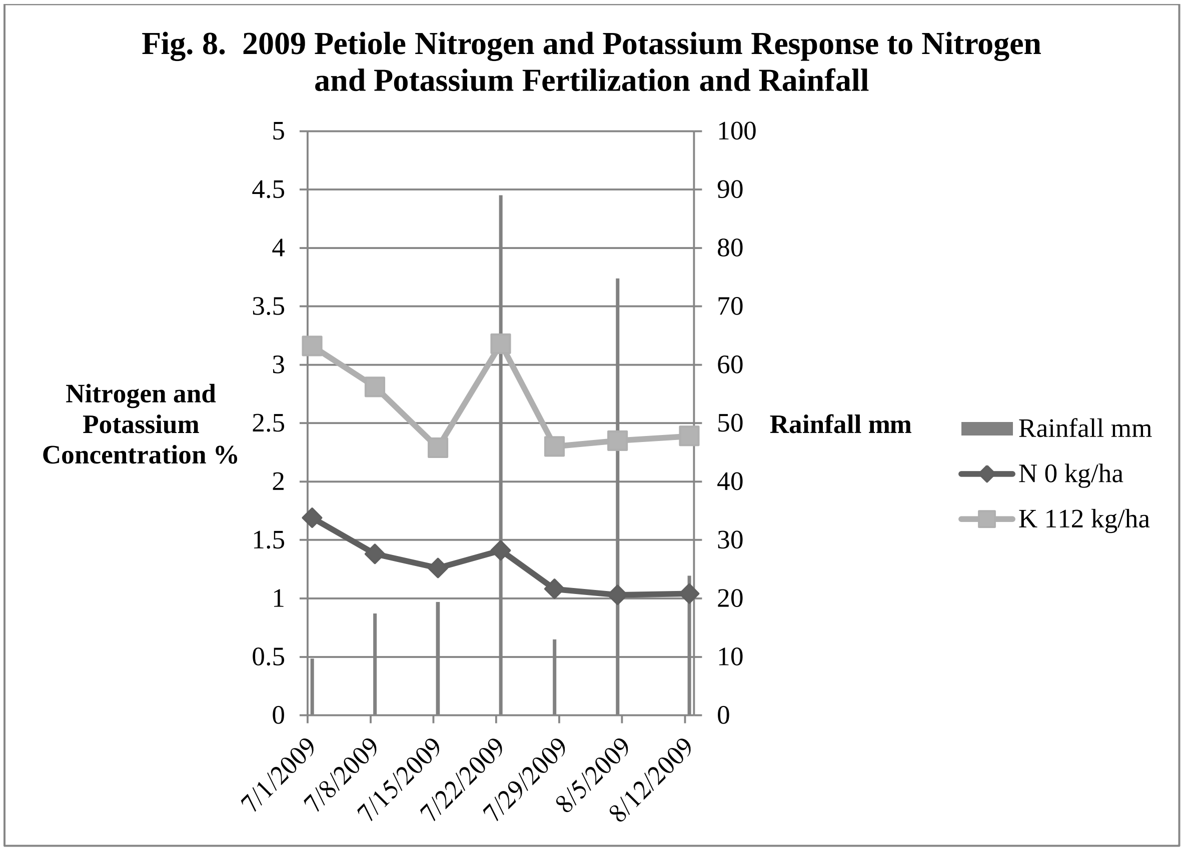

Figure 8. Response of petiole nitrogen and potassium concentration by sampling date beginning at match head square to one level of nitrogen (N) and potassium (K) fertilization at the Upper Coastal Plain Research Station in 2009.

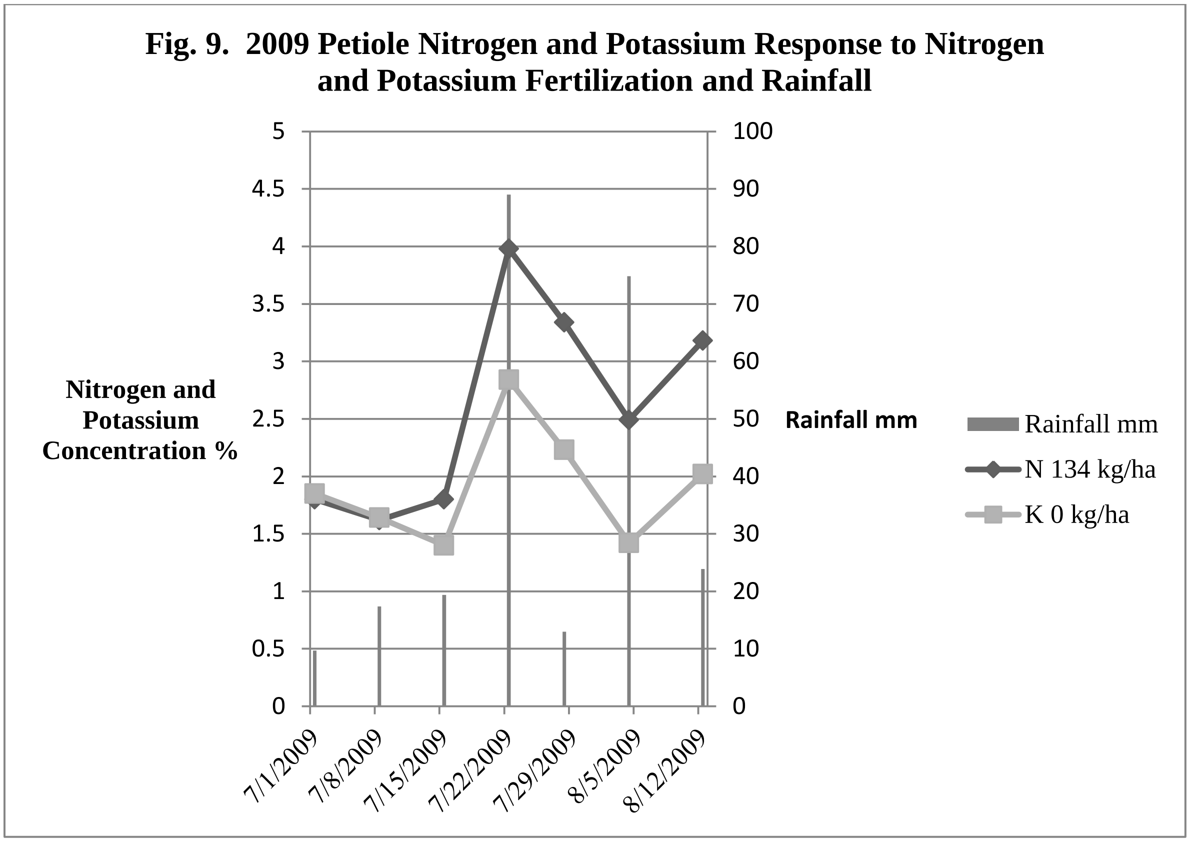

Figure 9. Response of petiole nitrogen and potassium concentration by sampling date beginning at match head square to one level of nitrogen (N) and potassium (K) fertilization at the Upper Coastal Plain Research Station in 2009.

APPENDIX

Table A.1. Response of leaf phosphorous concentration at various weeks starting at match head square to four levels of nitrogen fertilization.

Nitrogen	P Concentration			
	Weeks starting at match head square			
	4	6	7	8
kg/ha^{-1}	---------------------------------%---------------------------------			
		2008		
0	0.43	0.42	0.42	0.38
45	0.48	0.44	0.36	0.31
90	0.46	0.43	0.34	0.32
134	0.45	0.39	0.32	0.31
		2009		
0	0.35	0.30	0.37	0.35
45	0.34	0.34	0.35	0.37
90	0.33	0.35	0.37	0.36
134	0.34	0.36	0.34	0.35
		2010		
0	0.26	0.18	0.17	0.22
45	0.27	0.20	0.18	0.21
90	0.27	0.19	0.18	0.22
134	0.29	0.19	0.18	0.21
LSD (0.05)	0.03	0.03	0.03	0.03

†Match head square starts at week 1.

‡Weeks 4, 6, 7, and 8 represented the most significant response of leaf phosphorous concentration
to nitrogen fertilization.

§Leaf data was collected in 2008, 2009, and 2010.

Table A.2. Response of petiole phosphorous concentration at various weeks starting at match head square to four levels of nitrogen fertilization.

Nitrogen	P Concentration	
	Weeks starting at match head square	
	6	8
kg/ha^{-1}	---------------%--------------	
	2009	
0	0.20	0.20
45	0.25	0.25
90	0.25	0.24
134	0.25	0.23
	2010	
0	0.09	0.13
45	0.10	0.13
90	0.09	0.13
134	0.09	0.13
LSD (0.05)	0.38	0.31

†Match head square starts at week 1.

‡Weeks 6 and 8 represented the most significant response of petiole phosphorous concentration to nitrogen fertilization.

§Petiole data was collected in 2009 and 2010.

Table A.3. Response of petiole phosphorous concentration at various weeks starting at match head square to three levels of potassium fertilization.

Potassium	P Concentration
	Weeks starting at match head square
	3
kg/ha^{-1}	--%--
	2009
0	0.26
56	0.22
112	0.21
	2010
0	0.20
56	0.19
112	0.19
LSD (0.05)	0.28

†Match head square starts at week 1.
‡Week 3 represented the most significant response of petiole phosphorous concentration to potassium fertilization.
§Petiole data was collected in 2009 and 2010.

Table A.4. Response of petiole phosphorous concentration at various weeks starting at match head square to four levels of nitrogen and three levels of potassium fertilization.

Nitrogen	Potassium	P Concentration
		Weeks starting at match head square
		2
kg/ha^{-1}	kg/ha^{-1}	--%--
		2009
0	0	0.27
0	56	0.24
0	112	0.26
45	0	0.27
45	56	0.25
45	112	0.28
90	0	0.32
90	56	0.28
90	112	0.24
134	0	0.27
134	56	0.27
134	112	0.24
		2010
0	0	0.26
0	56	0.24
0	112	0.20
45	0	0.22
45	56	0.27
45	112	0.23
90	0	0.26
90	56	0.22
90	112	0.25
134	0	0.28
134	56	0.21
134	112	0.24
LSD (0.05)		0.69

†Match head square starts at week 1.
‡Week 2 represented the most significant response of petiole phosphorous concentration to combined treatments of nitrogen and potassium fertilization.
§Petiole data was collected in 2009 and 2010.

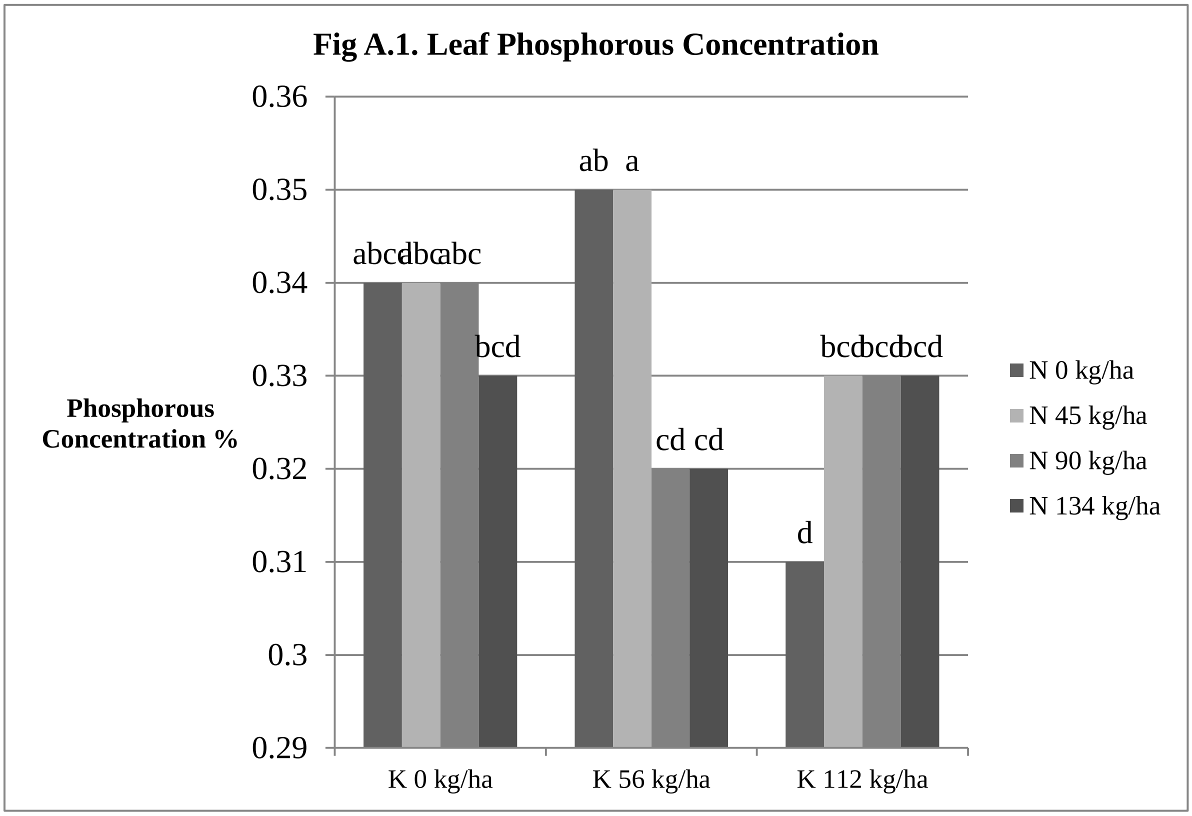

Figure A.1. Response of leaf phosphorous concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.

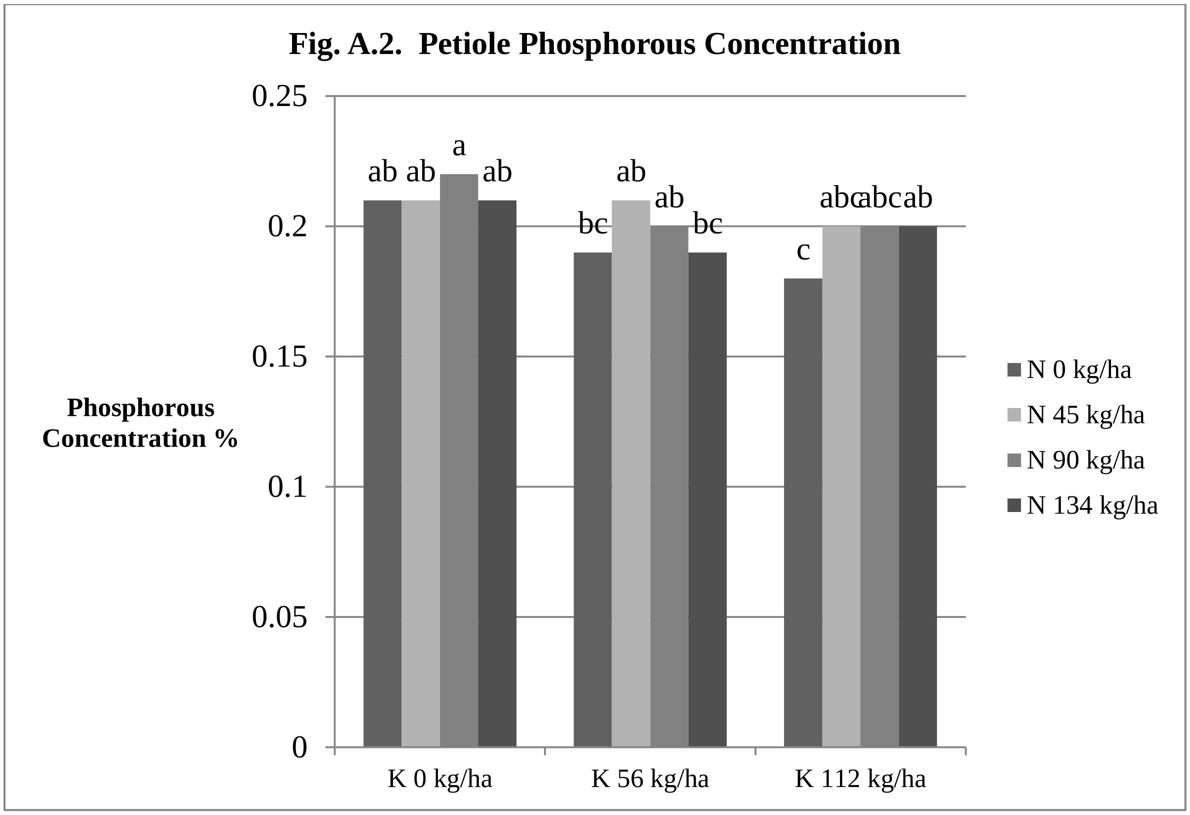

Figure A.2. Response of petiole phosphorous concentration to four levels of nitrogen (N) and three levels of potassium (K) fertilization (kg/ha) over all experiments.