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## Evaluation of technology aimed to improve nitrogen use efficiency for delayedlood rice (*Oryza sativa* L.) production

Jason Morris Satterfield

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EVALUATION OF TECHNOLOGY AIMED TO IMPROVE  
NITROGEN USE EFFICIENCY FOR DELAYED-FLOOD  
RICE (*Oryza sativa* L.) PRODUCTION

By

Jason Morris Satterfield

A Thesis  
Submitted to the Faculty of  
Mississippi State University  
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in Agronomy  
in the Department of Plant and Soil Sciences

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RICE (*Oryza sativa* L.) PRODUCTION

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Improving nitrogen (N) use efficiency is crucial for maximizing growth and yield in rice production. Tools to determine precise midseason N rates as well as knowledge of the effects of starter fertilizer N applications on rice growth and yield are lacking. Field experiments were conducted in 2007 and 2008 at the Delta Research and Extension Center to evaluate canopy reflectance as a means for determining yield and N nutrition at midseason and to assess agronomic effects and N recovery efficiency of starter fertilizer N.

Measurable in-season parameters were significantly related to grain yield. Grain yield and measured parameters were related to canopy reflectance. The results of this study support the continued research of canopy reflectance for predicting N nutrition indicators and yield. Minimal growth responses were observed when starter N was applied to seedling rice. Nitrogen recovery increased significantly throughout the growing season; however, less than 20% recovery was obtained.

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## CHAPTER I

### INTRODUCTION

Worldwide populations are continually increasing and so is the demand for rice (*Oryza sativa* L.). In 2008, United States rice production was near 1.21 million ha (NASS, 2009). Mississippi accounted for approximately 93,000 ha, a 21% increase from 2007 (Childs, 2009). The United States' rice hectarage is small compared with worldwide production. However, approximately 45% of rice produced each year in the United States is exported, making the United States the fourth largest exporter of rice (Childs, 2008). Worldwide stocks to use ratios have reached near record lows, increasing the market value of rice. Rice production costs have also increased dramatically. Cost increases are largely due to increases in the price of petroleum, thus driving fuel, fertilizer, and pesticide costs higher. Because of these escalating costs, increasing production efficiency is of paramount importance to growers. The development and implementation of advanced technological resources are critical for increasing efficiency and assuring future success of both United States and worldwide rice production.

#### Nitrogen Management in Rice

Nitrogen is the most limiting plant nutrient associated with rice production throughout the world, and rising N fertilizer costs have made it one of the highest

production expenses (Norman et al., 2003). In the United States, the price of N fertilizer has more than doubled since 2000, shifting from \$200 per ton to approximately \$550 per ton (NASS, 2009). This increase necessitates improving N fertilizer management in rice production. Development and evaluation of methods to improve N fertilizer management have not been stagnant; however, adoption of these methods has been slow and growers continue to apply midseason N in excess of needed requirements (Singh et al., 2002). Over fertilization with N can increase the incidence and severity of diseases and lodging, which in turn can decrease the economic return on rice either by increasing fungicide costs or decreasing harvest efficiency (Slaton et al., 2003; Slaton et al., 2004).

Nitrogen fertilizer is applied to five-leaf rice prior to flood establishment, referred to as a preflood application, and at midseason during panicle differentiation (PD). Panicle differentiation is when the uppermost internode elongates to a length of 1.3 to 2 cm. Midseason N applications are crucial during this time as the rice plant is transitioning from vegetative growth to reproductive growth. Research indicates that plant uptake of N fertilizer applied at midseason is slightly more efficient than preflood N, but preflood N has a much greater impact on the final grain yield of rice (Norman et al., 2003). Preflood N rates have been developed from empirical N rate studies based on cultivars grown, soil properties, environmental conditions, and crop history (Wells et al., 1989).

Greater than 70% of the total N requirement for rice can be accumulated during vegetative growth, which is characterized by active tillering, an increase in plant height, and leaf emergence at regular intervals (Norman et al., 2003). Midseason N rates are estimated, making precise N management decisions difficult. Due to the lack of

knowledge and resources to quickly and efficiently assess N status at midseason, N fertilizer application rates often exceed plant needs. Therefore, quantifying optimum midseason N fertilizer requirements is an important step toward developing an economically and environmentally viable crop production system (Varvel et al., 1997). For these reasons, development and implementation of rapid, reliable, non-destructive methods to monitor and determine plant N status at midseason are critical for precision N management strategies in rice.

Because improvement of N fertilizer use efficiency is crucial for rice production, numerous non-destructive tools have been evaluated for estimation of midseason N needs. The chlorophyll meter quantifies green color in plants by measuring chlorophyll status (Yadava, 1986), and estimations of N needs are represented by soil plant analytical division (SPAD) values. Soil plant analytical division values are dimensionless and represent a relative index of leaf chlorophyll and total N content (Huang and Peng, 2003). A high N reference strip must be developed in each field to determine critical SPAD values (Stevens et al., 2008). Turner and Jund (1991) found that 62% of the variability in rice yield response to midseason N could be explained by SPAD values of the most recently matured leaf. Similarly, Ntamatungiro et al. (1999) reported that 44 to 63% of variability associated with total N accumulation could be explained by SPAD values. The uniformity of SPAD values are affected by rice cultivar, leaf position on the plant, location on leaf where the reading is collected, and deficiencies of other nutrients (Turner and Jund, 1991). This variability in SPAD readings decreases the reliability of the chlorophyll meter for determining N fertilizer needs. Furthermore, few United States rice growers and crop consultants use chlorophyll meters for determining midseason N

requirements because of instrument cost and the need to establish high N reference strips to determine critical SPAD values (Stevens et al., 2008).

The rice gauge and yardstick method are non-destructive tools which use plant area measurements for predicting N needs. Plant area methods are based on the assumption that rice cultivars develop sufficient growth at specific, key growth stages to produce maximum grain yields (Ntamatungiro et al., 1999). The rice gauge utilizes horizontal and vertical rulers for measuring plant height and canopy width (Wells et al., 1989). Ntamatungiro et al. (1999) found that plant area determined with a rice gauge consistently explained up to 67% of the variability associated with determining midseason N needs. The yardstick method consists of placing a yardstick between two drill rows and counting the visible marks not obstructed by rice leaves (Stevens et al., 2008). Stevens et al. (2008) found that when 30 to 35 yardstick numbers were visible, rice was severely deficient in N; however, <13 visible numbers indicated no yield response from N application would be obtained. Plant area methods can be time consuming and labor intensive. Also, because these methods assume that a few measurements are representative of an entire field, accurately determining N status is difficult. Another disadvantage of using plant dry matter to estimate total N accumulation is the assumption that all other nutrients are in proper supply (Ntamatungiro et al., 1999). The use of plant area measurements is not practical with the need for timely, efficient midseason N applications.

The leaf color chart is a non-destructive tool that has been evaluated for predicting N nutrition of rice. Scientists at the International Rice Research Institute in the Philippines developed the leaf color chart for measuring rice leaf green color intensity

(Shukla et al., 2004). Leaf color charts estimate N needs based on visual observations of the rice plant. Singh et al. (2002) found that rice response to N fertilizer applications based on the leaf color chart was consistent with N fertilization applications based on readings of a chlorophyll meter. Problems with the leaf color charts include the need for high N reference strips and the inability of red-green color blind individuals to match a rice leaf to the green color plates on the charts (Stevens et al., 2008). Although numerous diagnostic tools have potential use in aiding N management decisions, the problems associated with each emphasizes the need for continued development of a more efficient and effective non-destructive resource.

### Remote Sensing Technologies

Remote-sensing techniques, including visible and infrared canopy reflectance measurements, can provide an instantaneous, nondestructive, and quantitative assessment of a crop's ability to intercept radiation and photosynthesize (Ma et al., 1996). A crop canopy's spectral reflectance is a combination of the plant and soil reflectance spectra, which are governed by the optical properties of these elements, and the radiant energy exchange within the canopy (Huete, 1988). As with other non-destructive tools, canopy spectral reflectance has been evaluated because growers recognize differences in productivity within individual fields and the potential value of variable rate fertilizer application technology (Shanahan et al., 2004). The canopy's spectral reflectance can be used to detect plant physiological and morphological differences (Johannsen and Sanders, 1982). Readings are collected at designated growth stages throughout the growing season and can provide input for developing management decisions. Canopy spectral



reflectance in the visible spectrum is inversely related to chlorophyll concentration, and canopy spectral reflectance in the near infrared portion (NIR) of the spectrum is directly related to the green leaf density (Gates et al., 1965; Knipling, 1970). Chlorophyll concentration can be used for estimation of N nutrition status because N is directly related to chlorophyll content.

Research has evaluated different vegetative indices derived from canopy spectral reflectance to measure specific parameters in corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean [*Glycine max* (L.) Merr.] (Ma et al., 2001; Moges et al., 2004; Sripada et al., 2005). Spectral reflectance indices were developed using simple mathematical formulas, such as ratios or differences between the reflectance at given wavelengths (Araus et al., 2001). Vegetative indices use specific wavelengths in the NIR (750 to 1300 nm) and visible portions (380 to 750 nm) of the spectrum to help identify the most significant relationships between the reflectance spectra and the parameters of interest. In the visible spectrum, the green (G) region (490 to 560 nm) and red (R) region (640 to 750 nm) are used in the green normalized difference vegetative index (GNDVI) and normalized difference vegetative index (NDVI), respectively. Sripada et al. (2005) found that spectral reflectance of corn expressed using GNDVI  $[(\text{NIR} - \text{G})/(\text{NIR} + \text{G})]$  relative to high-N reference strips successfully predicted optimum sidedress rates for pretassel N applications. GNDVI was reported to be a reliable predictor of wheat biomass, N uptake, grain yield, and grain N uptake (Moges et al., 2004). Ma et al. (2001) reported up to 80% of soybean grain yield could be explained by canopy reflectance expressed as NDVI  $[(\text{NIR} - \text{R})/(\text{NIR} + \text{R})]$ , measured non-destructively between the R4 and R5 growth stages.

Literature regarding the relationships between rice growth parameters and canopy spectral reflectance is not extensive. Rice canopy reflectance is of special interest due to varying canopy structures and the unique flooded environment in which rice is produced. Chang et al. (2005) reported that regression equations derived from canopy reflectance values measured at booting stage accurately predicted rice grain yield. Rice leaf area index and biomass could be predicted from spectral reflectance with  $r^2$  values of 0.67 and 0.97, respectively (Casanova et al., 1998). Xue et al. (2004) reported that rice canopy reflectance spectra were strongly correlated to leaf N concentration and leaf N accumulation.

Because plant physiological and morphological differences throughout the growing season are indicators of crop parameters, canopy spectral reflectance has potential to become a reliable, non-destructive resource for determining N nutrition status. The ability to apply the precise quantity of N required by the rice plant could optimize yield and prevent excessive application of N, which could have positive economical implications at the grower level. Furthermore, this technology could help alleviate environmental concerns such as eutrophication, caused by increased leaching and runoff of N fertilizer. The ability to make N fertilizer application decisions based on the entire crop canopy rather than individual plants is advantageous, and with advancements in variable rate fertilizer application technology, precision N applications based on crop needs could improve overall N use efficiency.

## Starter Fertilizer Nitrogen Applications

Starter N fertilizer applications are another area of interest for maximizing N use efficiency in rice. Numerous studies have evaluated starter N applications in soybean and corn (Vetsch and Randall, 2000; Bermudez and Mallarino, 2002; Niehues et al, 2004; Kaiser et al., 2005; Osborne and Riedell, 2006). In South Dakota, soybean biomass at V3 to V4 increased linearly with increasing fertilizer N rate up to 24 kg N ha<sup>-1</sup> when N was applied at planting in a band 5 cm beside and 5 cm below the seed (Osborne and Riedell, 2006). Corn dry weight measured at V5 to V6 was greater at 12 of 14 Iowa sites following a starter fertilizer application containing 3, 6, and 12 kg ha<sup>-1</sup> of N, P, and K, respectively (Kaiser et al., 2005). Early-season corn biomass increased 32% following starter fertilizer applied at planting (Bermudez and Mallarino, 2002). Niehues et al. (2004) found that starter N applications in continuous no-till corn increased early-season dry matter and significantly increased yields. Vetsch and Randall (2000) found that starter N injected below the soil surface consistently increased no-till corn yields.

Research on the effects of starter N applications to seedling rice is lacking. In Mississippi, most rice is planted on alluvial clay soils where seedling growth can be negatively affected by low availability of N. This low availability is enhanced in dry-seeded production where N fertilizer is normally not applied until the five-leaf rice growth stage. Typically, rice grown on clays soils requires 35 to 70 kg ha<sup>-1</sup> more N fertilizer compared to silt loam soils to achieve similar yields (Norman et al., 2005; Walker, 2006). Walker et al. (2008) found that starter N application of AMS (ammonium sulfate) or DAP (diammonium phosphate) increased plant height at five-leaf rice growth stage and moderately increased grain yield. Some potential positive impacts of starter N

applications in rice include the ability to have earlier flood establishment and potentially decrease herbicide applications for growers. Furthermore, earlier flood establishment increases the number of days rice vegetative growth occurs in a flooded environment, which has positive implications on nutrient availability and uptake as well as plant development (Walker et al., 2008). Knowledge of how rice utilizes N throughout the entire growing season could help strengthen overall N fertilizer use efficiency.

It is important to use reliable research techniques to better understand how starter N applications affect rice throughout the growing season. The use of tracer methods can provide direct measurement of N transformations (Hauck, 1982). The stable N isotope ( $^{15}\text{N}$ ) is a good tracer element for soil and plant N studies because of its known stability in long-term studies (Vose, 1980). In soil and plant studies,  $^{15}\text{N}$  is contained within plots using retainers to contain root growth and prevent  $^{15}\text{N}$  movement in the flood water and the soil solution (Bufogle et al., 1997). Advantages to tracer methodology include the positive identification of the labeled N entity as it may enter, be transformed within, or leave the system under study (Hauck and Bremner, 1976). Understanding plant N demand is a critical factor involved with N fertilization strategies for flooded rice, and with increasing concern for maximizing the efficiency of fertilizer N use in crop production, the use of  $^{15}\text{N}$  to study uptake of applied N could be beneficial.

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CHAPTER II  
ASSESSMENT OF RICE (*Oryza sativa* L.) YIELD AND NITROGEN NUTRITION  
STATUS USING CANOPY SPECTRAL REFLECTANCE

Abstract

Nitrogen input budgets for rice include a combination of the N source costs and its application. Southern United States rice production requires 150 to 200 kg N ha<sup>-1</sup>, which represents a significant portion of the total rice production budget. Small plot research techniques were employed in 2007 and 2008 at the Delta Research and Extension Center near Stoneville, MS, to evaluate the potential for using canopy spectral reflectance as a surrogate measurement for determining grain yield potential and components that are related to yield potential. Total dry matter (TDM), N concentration, total N uptake (TNU), NIR reflectance (rNIR<sub>850nm</sub>), GNDVI [(NIR – G)/(NIR + G)], and NDVI [(NIR – R)/(NIR + R)] were determined at panicle differentiation (PD) from plots where a series of N treatments ranging from 0 to 202 kg N ha<sup>-1</sup> had been applied at the five-leaf growth stage. Pearson's correlation coefficients (r) indicated that GY (grain yield) was positively related to N concentration, TDM, and TNU (r = 0.72 to 0.87). Furthermore, N concentration, TDM, TNU, and GY were positively related to spectral measurements (r = 0.58 to 0.95). Grain yield and TNU were similar in that as they related to GNDVI, NDVI, and rNIR<sub>850nm</sub> reflectance, both were best described by

exponential functions. Coefficients of determination ( $r^2$ ) ranged from 0.75 to 0.90 and 0.58 to 0.79 for GY and TNU, respectively. In most situations, the relationships between N concentration and spectral measurements were best described by exponential functions; however, greater variability existed ( $r^2 = 0.49$  to  $0.65$ ). Finally, TDM, when related to GNDVI and NDVI, was best described by exponential functions ( $r^2 = 0.42$  to  $0.85$ ); however, a quadratic function best described the relationship between TDM and  $rNIR_{850nm}$  ( $r^2 = 0.54$  to  $0.87$ ). Since canopy reflectance is strongly related to final GY and major yield contributing factors related to N nutrition, canopy spectral reflectance should be further investigated for its potential to determine the optimum N rate needed at PD to increase nitrogen use efficiency (NUE) and potentially provide greater economic returns while minimizing negative environmental effects.

### Introduction

In the absence of fertilization, N is a major limiting plant nutrient associated with rice production (Norman et al., 2003). Recently, increased N fertilizer costs have made it one of the major expenses associated with rice production. In the United States, the price of N fertilizer has been extremely volatile and reached a high of over \$800 per ton in the summer of 2008 compared to an average price of \$200 per ton paid by producers for the calendar year of 2000 (NASS, 2009). Though this price increase has greatly affected producers' production budgets, if the appropriate rate is not applied or efficiency is compromised, the costs associated with yield loss can far out-weigh the price of the product.

Rice production in Mississippi, Arkansas, Missouri, and much of Louisiana occurs in a drill-seeded, delayed-flood manner. Essentially, rice is grown as an upland crop through its seedling stage. At the onset of active tillering, which typically corresponds to the five-leaf growth stage, N fertilizer is applied as urea on dry soil just prior to flood establishment. Preflood applications are vital for vegetative growth which is essential to producing high grain yields, as more than 70% of the total N requirements for rice can be accumulated during the three to four weeks of vegetative growth following flood establishment (Norman et al., 2003). It is imperative that rice plants are not deficient in N during the reproductive growth stage because grain filling is also dependent on N and contributes greatly to yield. To ensure late-season deficiencies do not occur, it is common to apply N fertilizer during the early reproductive growth stage (midseason) between panicle initiation and panicle differentiation (PD). Preflood N rates are developed from empirical N rate studies based on cultivars grown, soil properties, environmental conditions, and crop history (Wells et al., 1989). Although preflood N rates have been established (Mengel and Wilson, 1988; Wilson et al., 1998; Walker, 2005) and optimum fertilizer N application timings have been identified (Bollich et al., 1994; Wilson et al. 1998; Walker et al., 2006), a method for determining precise midseason N rates based on plant needs is lacking.

The potential for N fertilizer application rates to exceed plant needs is due to the lack of knowledge and resources to quickly and efficiently assess N status at midseason. Growers have the tendency to over apply N at PD in hopes of ensuring maximum N uptake to obtain the highest yields possible. However, over fertilization with N can adversely affect growth by increasing the incidence and severity of diseases and lodging

which can decrease the economic return on rice either by increasing fungicide costs or decreasing harvest efficiency (Slaton et al., 2003; Slaton et al., 2004).

Numerous methods and tools have been evaluated over the years for assessing midseason N needs. Surrogate measurements for leaf N content have been evaluated for their effectiveness in determining the need for midseason N. The chlorophyll meter quantifies green color in plants by measuring chlorophyll status (Yadava, 1986), and estimations of N needs are represented by soil plant analytical division (SPAD) values. SPAD values are dimensionless and represent a relative index of leaf chlorophyll and total N contents (Huang and Peng, 2003). A high N reference strip must be developed in each field for determination of critical SPAD values (Stevens et al., 2008). The uniformity of SPAD values are affected by rice cultivar, leaf position on the plant, location on leaf where the reading is collected, and deficiencies of other nutrients which decrease the reliability of this tool for determining the need for N fertilizer (Turner and Jund, 1991). Scientists at the International Rice Research Institute in the Philippines developed the leaf color chart for measuring rice leaf green color intensity (Shukla et al., 2004). Leaf color charts estimate N needs based on visual observations of the rice plant. Problems with the leaf color charts include the need for high N reference strips and the inability of red-green color blind individuals to match a rice leaf to the green color plates on the charts (Stevens et al., 2008).

Measurements based on biomass have also been tested for their effectiveness in prescribing midseason N fertilization. The rice gauge utilizes horizontal and vertical rulers for measuring plant height and canopy width (Wells et al., 1989). Stevens et al. (2008) evaluated a low-cost method which estimates N need based on rice canopy

closure. This method includes placing a yardstick between two drill rows and counting the visible marks not obstructed by rice leaves (Stevens et al., 2008). Plant area methods are based on the assumption that rice cultivars develop sufficient growth at specific stages to produce maximum grain yields (Ntamatungiro et al., 1999). The use of plant area measurements can be time consuming and labor intensive and are not practical with the need for timely, efficient midseason N applications. Although numerous diagnostic tools have been developed for aiding N management decisions, the problems associated with each emphasizes the need for continued development of a more efficient and effective non-destructive resource.

Remote-sensing techniques, including visible and infrared canopy reflectance measurements, can provide an instantaneous, nondestructive, and quantitative assessment of a crop's ability to intercept radiation and photosynthesize (Ma et al., 1996). A crop canopy's spectral reflectance is a combination of the plant and soil reflectance spectra, which are governed by the optical properties of these elements, and the radiant energy exchange within the canopy (Huete, 1988). Readings are collected at designated growth stages throughout the growing season and can provide input for developing management decisions.

Research has evaluated different vegetative indices derived from canopy spectral reflectance to measure specific parameters in corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean [*Glycine max* (L.) Merr.] (Ma et al., 2001; Moges et al., 2004; Sripada et al., 2005). Vegetative indices use specific wavelengths in the NIR (750 to 1300 nm) and visible portions (400 to 750 nm) of the spectrum to help identify the most significant relationships between the reflectance spectra and the parameters of interest.

In the visible spectrum, the green (G) region (495 to 570 nm) and red (R) region (620 to 750 nm) are used in the green normalized difference vegetative index (GNDVI) and normalized difference vegetative index (NDVI), respectively. Sripada et al. (2005) found that spectral reflectance of corn expressed using GNDVI  $[(\text{NIR} - \text{G})/(\text{NIR} + \text{G})]$  relative to high-N reference strips successfully predicted optimum sidedress rates for pretassel N applications. Moges et al. (2004) reported GNDVI to be a reliable predictor of wheat biomass, N uptake, grain yield, and grain N uptake. Ma et al. (2001) reported up to 80% of soybean grain yield could be explained by canopy reflectance expressed as NDVI  $[(\text{NIR} - \text{R})/(\text{NIR} + \text{R})]$ , measured non-destructively between the R4 and R5 growth stages.

Literature regarding the relationship between rice growth parameters and canopy spectral reflectance is not extensive. Rice canopy reflectance is of special interest due to varying canopy structures and the unique flooded environment in which rice is produced. Chang et al. (2005) reported that regression equations derived from canopy reflectance values measured at booting stage accurately predicted rice grain yield. Xue et al. (2004) reported that rice canopy reflectance spectra were strongly correlated to leaf N concentration and leaf N accumulation.

The objective of this research was to evaluate canopy spectral reflectance as a non-destructive measurement to assess rice grain yield and N nutrition at midseason.

## Materials and Methods

### *Site Description and Cultural Practices*

Field studies were conducted at the Delta Research and Extension Center near Stoneville, MS, in 2007 and 2008, on Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) soil with a pH of 8.0, organic matter content of 22 g kg<sup>-1</sup>, and a CEC of 42 cmol<sub>c</sub> kg<sup>-1</sup>. Three rice cultivars {'Cocodrie' (Linscombe et al., 2000), 'Wells' (Moldenhauer et al., 2001), and 'XL723' (RiceTec, Inc., Alvin, TX)} were drill-seeded adjacent to each other but within the same paddy. Cocodrie and Wells were planted at 90 kg ha<sup>-1</sup>, and 'XL723' was planted at 35 kg ha<sup>-1</sup>, which are common seeding rates for the two inbred cultivars and F<sub>1</sub> hybrid, respectively. Rice was grown in an upland condition until the five-leaf growth stage. When rice reached the five-leaf growth stage, N fertilizer treatments (0, 67, 101, 134, 168, and 202 kg N ha<sup>-1</sup>) were randomly applied to experimental units that consisted of eight drill rows spaced 20 cm apart and measuring 4.6 m in length. All replications were separated by 1.6 m alleys. Granular urea (460 g N kg<sup>-1</sup>) was pre-weighed into polypropylene bottles and distributed onto dry soil two days prior to flood establishment with a custom-manufactured, self-propelled distributor equipped with a Hege 80 belt cone (Wintersteiger, Inc., Salt Lake City, UT) and a zero-max (Zero-Max, Inc., Plymouth, MN) to ensure accuracy and precision. Plots were harvested when grain moisture ranged from 150 to 180 g kg<sup>-1</sup> with a Wintersteiger Delta Combine (Wintersteiger, Inc., Salt Lake City, UT) equipped with a Harvest Master Grain Gauge (Juniper Systems, Inc., Logan, UT) for measuring weight and moisture. Prior to statistical analyses, grain yields (GY) were standardized to 120 g kg<sup>-1</sup> moisture content.

### *Data Collection*

A handheld GER 1500 spectroradiometer (INSTAAR, Inc., Clemson, SC) was used to collect canopy reflectance readings from each plot at a distance of approximately 0.80 m above the canopy. Canopy spectral reflectance was measured between the hours of 1000 and 1400 on days with minimal cloud cover to minimize changes in the sun's angle and shadowing effects. The GER 1500 has 512 channels and measures in wavelengths of 1.5 nm increments from 350 to 1050 nm. Five canopy measurements of spectral radiance were randomly collected from each plot at PD. The subsequent radiance measurements were averaged to obtain the canopy spectral reflectance values for each plot. Reflectance at a single NIR waveband ( $rNIR_{850nm}$ ), and two vegetative indices based on reflectance at NIR and visible wavelengths were used for analyses. Specifically, the two indices included a normalized difference vegetative index (NDVI), where  $NDVI = (850nm - 650nm)/(850nm + 650nm)$ , and a green normalized difference vegetative index (GNDVI), where  $GNDVI = (850nm - 550nm)/(850nm + 550nm)$ .

Whole aboveground biomass was harvested from 0.9 m of linear row from the second row of each plot immediately following reflectance data collection. The tissue samples were oven-dried at 60°C until a constant weight was obtained (48 to 72 hr) and weighed to determine total dry matter (TDM). After being ground through a Wiley Mill, 4 to 6 mg of each sample were analyzed for N concentration using a Carlo Erba N/C 1500 dry combustion analyzer (Carlo Erba, Milan, Italy). Total N uptake (TNU) was the product of TDM and N content.



### *Statistical Analysis*

Pearson's correlation coefficients were determined using PROC CORR in SAS (2003) to verify that TNU, N concentration, and TDM were related to GY. Correlations between spectral reflectance indices ( $r_{\text{NIR}_{850\text{nm}}}$ , GNDVI, and NDVI) and TNU, N concentration, TDM and GY were also determined. Regression analysis was conducted using PROC GLM and PROC NLIN to quantify the relationships that were apparent from the correlation analyses. A quadratic regression model fit using the GLM procedure in SAS was used to evaluate the relationships between  $r_{\text{NIR}_{850\text{nm}}}$ , GNDVI, NDVI, and TDM, N concentration, TNU, and GY. To determine if the relationship was consistent across years, interaction terms between year and the linear coefficient and between year and the quadratic coefficient were included in the model as was a year effect. These terms allowed the intercepts, linear terms, and quadratic terms of the quadratic regression model to vary across years. If these terms were not significantly different from zero, then a model that assumed constant coefficients across years was fit.

### Results and Discussion

In order for canopy spectral reflectance to have potential as a tool to assess the N nutrition status of rice at midseason, sensitivity to reflectance differences must be present. For each cultivar, which represent three different canopy types, reflectance was highest in the NIR band (750 to 1300 nm) and reflectance was positively related to the N rate applied (Figures 2.1 to 2.3). Reflectance was high in this region due to well-developed microcellular leaf structures which cause scattering (Knipling, 1970). Since rice biomass, N concentration, and leaf area index are highly responsive to N fertilization, differences

in the reflectance spectra were expected. Furthermore, reflectance was negatively related to N rate in the visible region of the spectrum (400 to 750 nm) (Figures 2.1 to 2.3). These differences in N rate were most noticeable in the green band of the visible spectrum (495 to 570 nm). Chlorophyll absorbs light and its concentration is directly related to N concentration (Knipling, 1970; Hatfield et al., 2008); therefore, plots receiving higher N fertilization rates experienced more absorption in the visible spectrum. Xue et al. (2004) reported similar results in which reflectance decreased in the visible region with increasing N supply and increased in the NIR region, also where the most separation between N treatments was most noticeable. For all cultivars, sensitivity to changes in biomass, a function of N rate, was greatest at the wavelength range of 810 to 890 nm. A saturation effect with N rate was noted for Wells, where no increases in reflectance were observed above the 168 kg N ha<sup>-1</sup> rate (Figure 2.2).

Correlations were determined among GY and each of three parameters that were measured at PD which influence rice GY (N concentration, TNU, and TDM). Pearson's correlation coefficients (*r*) demonstrated that nitrogen concentration, TNU, and TDM were positively related to GY for all three cultivars ( $r \geq 0.72$ ) (Table 2.1). Since all measurable parameters were related to GY, correlations between N concentration, TNU, TDM, and GY and canopy spectral reflectance at PD were determined. All yield components measured at PD, as well as GY, were correlated ( $r \geq 0.58$ ) with reflectance indices for all cultivars (Table 2.2 to 2.4). It is important to note that TDM was most highly correlated with  $rNIR_{850nm}$  for all cultivars. The NIR portion of the spectrum is highly related to reflectance of leaf cellular structures, providing reason why this single wavelength may have correlated more strongly with TDM than the vegetative indices

which also account for other plant characteristics. For Cocodrie and Wells, GY was more strongly correlated with GNDVI and NDVI rather than  $rNIR_{850nm}$ . This may be due to the fact that GNDVI and NDVI account for regions of the spectrum where both biomass and N content are relevant. XL723, however, showed similar correlations between GY and  $rNIR_{850nm}$ , GNDVI, and NDVI ( $r = 0.87, 0.87, \text{ and } 0.83$ , respectively). Nitrogen concentration had the lowest correlation coefficients for all three cultivars.

Regression coefficients were considered constant across years for most of the measured parameters for each cultivar. Regression analyses resulted in significant exponential and quadratic relationships between all measured parameters and reflectance. For XL723, regression coefficients were constant for all parameters both years, so these were pooled together (Table 2.5). Furthermore, all parameters except TDM were best described exponentially for XL723. Total dry matter of XL723 was best described by a quadratic relationship when related to reflectance at  $rNIR_{850nm}$ . XL723 showed the greatest variability of the three cultivars ( $r^2 = 0.42 \text{ to } 0.82$ ). The variability of these results could be related to the lower plant density for hybrid cultivars, which may have affected the canopy spectral radiance collected at PD. GNDVI calculations did explain 82% of the variability associated with GY for XL723. Moges et al. (2004) reported a similar strong relationship between GNDVI and final grain yield in winter wheat ( $r^2 = 0.74$ ).

For Cocodrie, half of the twelve relationships were pooled across years while the rest were analyzed individually for 2007 and 2008. Total dry matter was best explained by a quadratic relationship with  $rNIR_{850nm}$  ( $r^2 = 0.87$ ). In 2007 and 2008, vegetative indices GNDVI and NDVI explained 88 and 90%, respectively, of the variability

associated with Cocodrie GY. Total N uptake in 2007 and 2008 was best explained by exponential relationships with GNDVI ( $r^2 = 0.79$  and  $0.82$ , respectively). Similarly, Xue et al. (2004) found that using a ratio index of NIR to G resulted in a highly significant relationship with rice N accumulation ( $r^2 = 0.85$ ).

For Wells, eight of the relationships were pooled across years while four were analyzed by year (Table 2.8 and Table 2.9). As with XL723 and Cocodrie, GY of Wells was best explained by GNDVI ( $r^2 = 0.90$ ). In 2007, GNDVI and NDVI both explained TNU with similar results ( $r^2 = 0.90$  and  $0.89$ , respectively). The same trend was observed in 2008 for the relationships between TNU with GNDVI and NDVI ( $r^2 = 0.80$  and  $0.81$ , respectively). Comparable results reported by Moges et al. (2004) for winter wheat showed that GNDVI explained up to 90% of N uptake. As stated earlier, Wells experienced a saturation effect in the NIR region when the  $168 \text{ kg N ha}^{-1}$  rate was applied. This may be an indication that using vegetative indices could be superior to a single wavelength when relating reflectance to N indicators. This is true because vegetative indices can account for more than one characteristic wavelength. Similar to the correlation statistics, a common trend shown in the regression analyses was that TDM was generally best explained by the single wavelength ( $r_{\text{NIR}_{850\text{nm}}}$ ) in the NIR region. Babar et al. (2006) found that using NIR-based indices consistently demonstrated higher levels of association with biomass production in wheat and explained a higher proportion of the variability.

## Conclusion

Rice grain yield is highly dependent on N uptake and biomass, much of which occurs during active vegetative growth stages. Canopy spectral reflectance was strongly correlated to major yield components as well as final grain yield for each of three popular rice plant types grown in the southern United States rice-producing region. Thus, the investigation of this technology to be used as a tool to more precisely determine the need for N fertilizer application during the onset of reproductive growth is warranted. This technology has potential to aid in improving N use efficiency and reduce the negative agronomic and environmental effects caused by over-fertilization. Future studies should incorporate different cultivars and be performed in other rice-growing regions where soil types and production practices differ.

Table 2.1 Pearson correlation coefficients for relationships among grain yield and N nutrition indicators {N concentration (%N), total N uptake (TNU), and total dry matter (TDM)} for ‘Cocodrie’, ‘Wells’, and ‘XL723’ grown at DREC in 007 and 2008.

	Yield		
	Cocodrie	Wells	XL723
N indicator	†r		
% N	0.77	0.79	0.80
TNU	0.73	0.81	0.87
TDM	0.72	0.76	0.82

†All relationships were significant at  $P < 0.0001$ .

Table 2.2 Pearson correlation coefficients for relationships among  $rNIR_{850nm}$ , GNDVI, and NDVI and the N nutrition indicators {total dry matter (TDM), total N uptake (TNU), N concentration (%N), and grain yield} for ‘Cocodrie’ grown at DREC in 2007 and 2008.

	TDM	TNU	%N	Yield
Vegetative Index	†r			
$rNIR_{850nm}$	0.93	0.87	0.68	0.77
GNDVI	0.77	0.70	0.66	0.91
NDVI	0.73	0.64	0.58	0.85

†All relationships were significant at  $P < 0.0001$ .

Table 2.3 Pearson correlation coefficients for relationships among the single wavelength and vegetative indices (rNIR<sub>850nm</sub>, GNDVI, and NDVI) and the N nutrition indicators {total dry matter (TDM), total N uptake (TNU), N concentration (%N), and grain yield} for ‘Wells’ grown at DREC in 2007 and 2008.

	TDM	TNU	%N	Yield
Vegetative Index	<sup>†</sup> r			
rNIR <sub>850nm</sub>	0.87	0.84	0.61	0.84
GNDVI	0.78	0.78	0.71	0.95
NDVI	0.79	0.77	0.68	0.92

<sup>†</sup>All relationships were significant at P < 0.0001.

Table 2.4 Pearson correlation coefficients for relationships among the rNIR<sub>850nm</sub>, GNDVI, and NDVI and the N nutrition indicators {total dry matter (TDM), total N uptake (TNU), N concentration (%N), and grain yield} for ‘XL723’ grown at DREC in 2007 and 2008.

	TDM	TNU	%N	Yield
Vegetative Index	<sup>†</sup> r			
rNIR <sub>850nm</sub>	0.71	0.76	0.71	0.87
GNDVI	0.70	0.72	0.68	0.87
NDVI	0.63	0.66	0.65	0.83

<sup>†</sup>All relationships were significant at P < 0.0001.

Table 2.5 Regression equations relating TDM, GY, TNU and %N to NDVI, GNDVI and rNIR<sub>850nm</sub> for 'XL723' when data were pooled across 2007 and 2008.

Vegetative Index	XL723	†R <sup>2</sup>
NDVI	$TNU_{XL723} = 3.365e^{3.720x}$	0.58
	$TDM_{XL723} = 13.06e^{1.916x}$	0.42
	$\%N_{XL723} = 0.475e^{1.81x}$	0.53
	$Yield_{XL723} = 1309e^{2.479x}$	0.75
GNDVI	$TNU_{XL723} = 3.629e^{4.209x}$	0.66
	$TDM_{XL723} = 12.95e^{2.236x}$	0.52
	$\%N_{XL723} = 0.517e^{1.981x}$	0.57
	$Yield_{XL723} = 1448e^{2.732x}$	0.82
rNIR <sub>850nm</sub>	$TNU_{XL723} = 8.464e^{0.056x}$	0.67
	$TDM_{XL723} = -0.038(rNIR_{850nm})^2 + 4.198(rNIR_{850nm}) - 34.666$	0.54
	$\%N_{XL723} = 0.762e^{0.026x}$	0.58
	$Yield_{XL723} = 2614e^{0.035x}$	0.78

†All regression equations were significant at P < 0.0001.



Table 2.6 Regression equations relating TDM, GY, TNU and %N to GNDVI and rNIR<sub>850nm</sub> for ‘Cocodrie’ when data were pooled across 2007 and 2008.

Vegetative Index	Cocodrie	†R <sup>2</sup>
GNDVI	$TDM_{Cocodrie} = 9.955e^{2.598x}$	0.76
	$Yield_{Cocodrie} = 2082e^{1.959x}$	0.88
rNIR <sub>850nm</sub>	$TNU_{Cocodrie} = 8.825e^{0.052x}$	0.79
	$TDM_{Cocodrie} = 0.01(rNIR_{850nm})^2 + 1.421(rNIR_{850nm}) + 1.479$	0.87
	$\%N_{Cocodrie} = 0.953e^{0.017x}$	0.49
	$Yield_{Cocodrie} = -6.308(rNIR_{850nm})^2 + 598.053(rNIR_{850nm}) - 4037.918$	0.76

†All regression equations were significant at P < 0.0001.

Table 2.7 Regression equations relating TNU, TDM, %N and GY with NDVI and GNDVI for ‘Cocodrie’ in 2007 and 2008.

Year	Vegetative Index	Cocodrie	†R <sup>2</sup>
2007	NDVI	$TNU_{Cocodrie} = 0.133e^{7.211x}$	0.75
		$TDM_{Cocodrie} = 0.927e^{5.018x}$	0.85
		$\%N_{Cocodrie} = 81.07(rNIR_{850nm})^2 - 125.224(rNIR_{850nm}) + 48.905$	0.59
		$Yield_{Cocodrie} = 130362.858(rNIR_{850nm})^2 - 189795.019(rNIR_{850nm}) + 74199.044$	0.89
	GNDVI	$TNU_{Cocodrie} = 0.864e^{5.94x}$	0.79
		$\%N_{Cocodrie} = 40.123(rNIR_{850nm})^2 - 49.312(rNIR_{850nm}) + 15.95$	0.67
2008	NDVI	$TNU_{Cocodrie} = 6.908e^{2.572x}$	0.79
		$TDM_{Cocodrie} = 16.23e^{1.514x}$	0.84
		$\%N_{Cocodrie} = 0.791e^{1.057x}$	0.60
		$Yield_{Cocodrie} = 18874.918(rNIR_{850nm})^2 - 10356.767(rNIR_{850nm}) + 4931.777$	0.90
	GNDVI	$TNU_{Cocodrie} = 6.388e^{3.064x}$	0.82
		$\%N_{Cocodrie} = 0.752e^{1.287x}$	0.65

†All regression equations were significant at P < 0.0001.

Table 2.8 Regression equations relating TDM, GY, TNU and %N to GNDVI and rNIR<sub>850nm</sub> for ‘Wells’ when data were pooled across 2007 and 2008.

Vegetative Index	Wells	†R <sup>2</sup>
NDVI	$TDM_{Wells} = 11.85e^{2.298x}$	0.79
	$Yield_{Wells} = 2339e^{1.59x}$	0.89
GNDVI	$TDM_{Wells} = 11.17e^{2.684x}$	0.76
	$\%N_{Wells} = 0.801e^{1.157x}$	0.62
	$Yield_{Wells} = 2180e^{1.904x}$	0.90
rNIR <sub>850nm</sub>	$TNU_{Wells} = 8.399e^{0.068x}$	0.75
	$TDM_{Wells} = -0.0135(rNIR_{850nm})^2 + 3.622(rNIR_{850nm}) - 19.247$	0.75
	$Yield_{Wells} = -8.119(rNIR_{850nm})^2 + 638.586(rNIR_{850nm}) - 3120.915$	0.80

†All regression equations were significant at P < 0.0001.

Table 2.9 Regression equations relating TNU and %N with NDVI, GNDVI, and rNIR<sub>850nm</sub> for 'Wells' in 2007 and 2008.

Year	Vegetative Index	Wells	†R <sup>2</sup>
2007	NDVI	$TNU_{Wells} = 3.214e^{3.993x}$	0.89
		$\%N_{Wells} = 9.76(rNIR_{850nm})^2 - 11.185(rNIR_{850nm}) + 4.29$	0.65
	GNDVI	$TNU_{Wells} = 3.743e^{4.369x}$	0.90
	rNIR <sub>850nm</sub>	$\%N_{Wells} = 0.844e^{0.021x}$	0.74
2008	NDVI	$TNU_{Wells} = 7.104e^{2.747x}$	0.81
		$\%N_{Wells} = 0.852e^{1.031x}$	0.68
	GNDVI	$TNU_{Wells} = 6.06e^{3.295x}$	0.80
	rNIR <sub>850nm</sub>	$\%N_{Wells} = 0.865e^{0.028x}$	0.53

†All regression equations were significant at P < 0.0001.

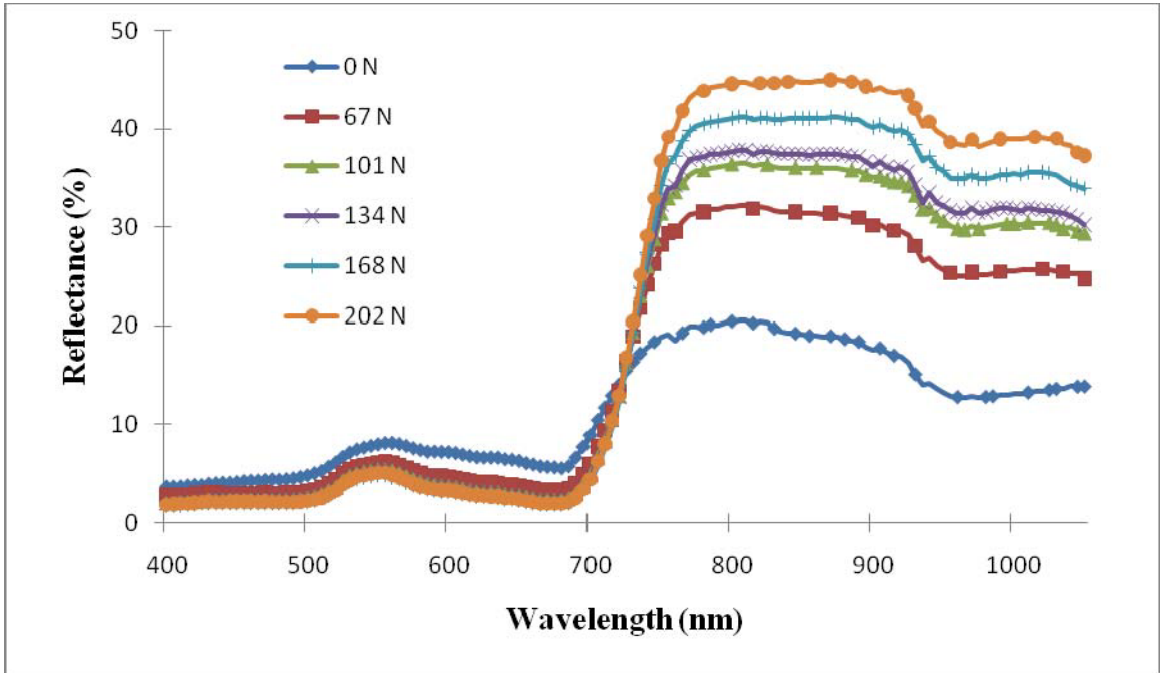


Figure 2.1 ‘Cocodrie’ canopy spectral reflectance response to PF N rates ranging from 0 to 202 kg N ha<sup>-1</sup> at the PD growth stage in 2007 and 2008.

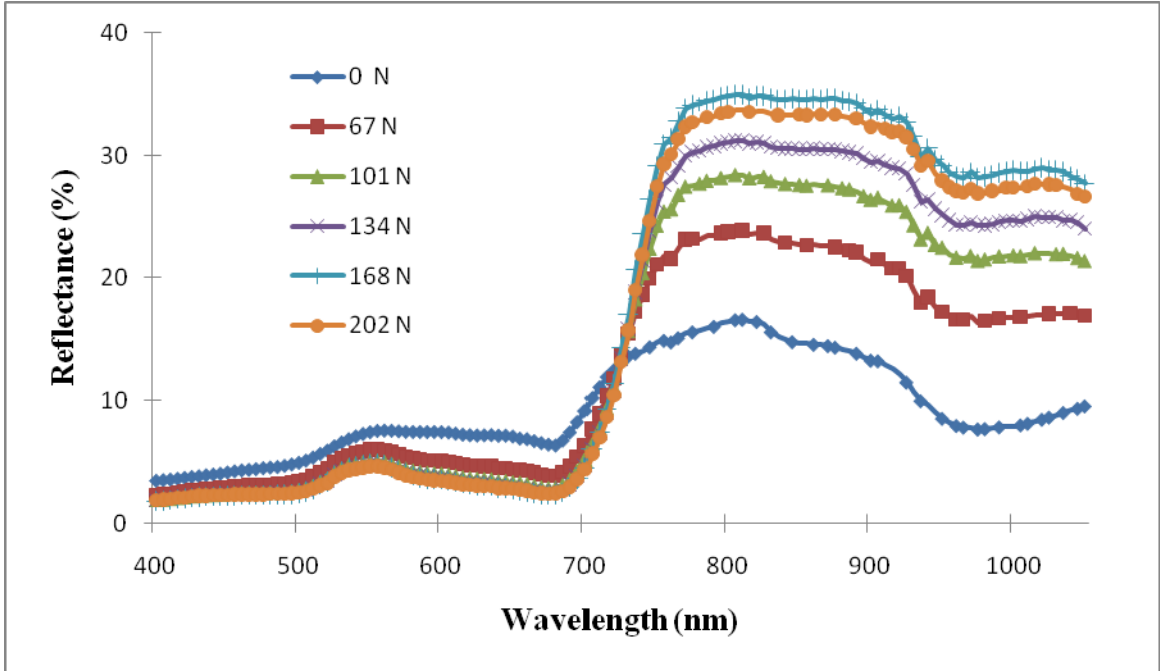


Figure 2.2 ‘Wells’ canopy spectral reflectance response to PF N rates ranging from 0 to 202 kg N ha<sup>-1</sup> at the PD growth stage in 2007 and 2008.

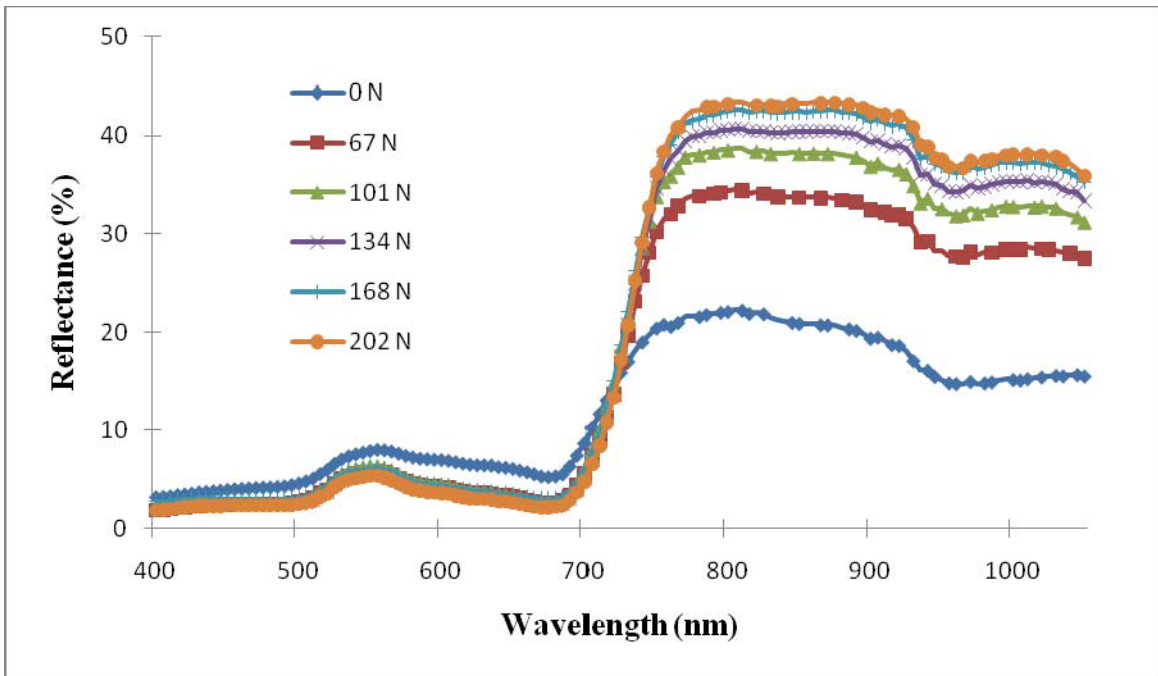


Figure 2.3 'XL723' canopy spectral reflectance response to PF N rates ranging from 0 to 202 kg N ha<sup>-1</sup> at the PD growth stage in 2007 and 2008.

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## CHAPTER III

### EFFECTS OF AMMONIUM SULFATE ON EARLY-SEASON RICE (*Oryza sativa* L.) GROWTH, GRAIN YIELD, AND NITROGEN RECOVERY

#### Abstract

Seedling rice growth on high CEC, montmorillonitic soils, representative of the majority of rice hectareage in Mississippi, can be slow. Research evaluated the effects of starter fertilizer application {ammonium sulfate (AMS)} on seedling growth and grain yield (GY) for two rice cultivars ('Cocodrie' and 'XL723') on a Sharkey (very fine, smectitic, thermic, Chromic Epiaquerts) clay soil in 2007 and 2008 at the Delta Research and Extension Center near Stoneville, MS. Starter AMS resulted in no increases in plant height or GY. Total dry matter (TDM) was increased 28% in 2008 when starter N was applied. Total N uptake (TNU) was also greater in 2008 when starter N was applied. In 2008, plant recovery of <sup>15</sup>N-AMS was approximately 3.5 times greater than in 2007 when measured at the five-leaf stage. The recovery percentage of <sup>15</sup>N-AMS reached its maximum at heading (HD) in both years. Maximum recovery averaged approximately 15% in 2007 and 8% in 2008. The hybrid XL723 had approximately 1% greater N concentration at the five-leaf rice growth stage, produced approximately 25% greater yield than Cocodrie in both years, and in 2007, recovered the greatest amount of starter N, thus providing evidence that heterosis is a means by which greater GY and N use

efficiency can be obtained. This study revealed that to more consistently increase growth and GY, studies should be conducted that investigate more effective starter fertilizer application strategies, including starter fertilizer N source, rate, and placement in proximity to the root zone.

## Introduction

A large percentage of rice hectareage in the Mississippi River Alluvial Floodplain is produced on high CEC, montmorillonitic clay soils where ammonium diffusion constraints can minimize N uptake (Trostle et al., 1998). Furthermore, semidwarf rice cultivars have become popular, especially in Mississippi, due to their ability to utilize relatively high rates of N which leads to greater grain yields while minimizing lodging potential (McClung, 2003). One negative of the semidwarf cultivars, which have shorter mesocotyl lengths, is that seedling vigor can be compromised (Turner et al., 1982). Soil environmental conditions commonly undergo extreme changes during seedling growth. These changes exacerbate problems with the inherently lower seedling vigor of semidwarf cultivars and low plant populations commonly used for hybrid cultivars. After stand establishment, many field observations have led growers, consultants, researchers, and extension personnel to believe that seedling growth on clay soils can be slow, especially for semidwarf and hybrid cultivars. Since rice seedling growth and development dictate the timing of preflood fertilizer N applications in delayed-flood rice culture, it is important to determine ways to enhance seedling growth.

Previous research has examined starter fertilizer application in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (Vetsch and Randall, 2000; Niehues et al, 2004;

Kaiser et al., 2005; Osborne and Riedell, 2006). In South Dakota, V3 to V4 soybean biomass increased linearly with increasing N rate up to 24 kg N ha<sup>-1</sup> when N was applied at planting in a band 5 cm beside and 5 cm below the seed (Osborne and Riedell, 2006). In Iowa, corn dry weight measured at V5 to V6 was greater following a starter fertilizer application containing 3, 6, and 12 kg ha<sup>-1</sup> of N, P, and K respectively (Kaiser et al., 2005). Niehues et al. (2004) found that starter N applications in continuous no-till corn increased early-season dry matter and yields. Vetsch and Randall (2000) found that starter N injected below the soil surface consistently increased no-till corn yields.

Increasing plant height by applying starter N could have many potential impacts on seedling rice, including an allowance for earlier pre-flood N applications and flood establishment. Walker et al. (2008) found that starter N application of AMS (ammonium sulfate) or DAP (diammonium phosphate) increased plant height by 3 cm at five-leaf rice. Because the flooded environment in which rice is produced can substantially impact nutrient availability and uptake, as well as weed control (Norman et al., 2003; Kendig et al., 2003), earlier flood establishment would be beneficial. Benefits would include increasing the number of days in which rice vegetative growth occurred in a flooded environment and possibly reducing herbicide usage which would save growers money (Walker et al., 2008).

Because N can undergo many transformations in both soil and plants, it is important to use fundamentally sound research techniques to account for these changes. The use of tracer methods can provide direct measurement of N transformations (Hauck, 1982). Tracer N methods give positive evidence that the labeled material has interacted in some manner with other nitrogenous constituents in the sample of interest (Hauck,

1982). Specific advantages to the use of tracer methods are the positive identification of the labeled N entity as it may enter, be transformed within, or leave the system under study (Hauck and Bremner, 1976). <sup>15</sup>Nitrogen-labeled isotopes have been used as tracer elements in soil and plant N studies because of their known stability in long-term studies (Vose, 1980). In soil and plant studies, the <sup>15</sup>N is generally contained within plots using retainers to contain root growth and prevent <sup>15</sup>N movement in the flood water and the soil solution (Bufogle et al., 1997).

While the impacts of preflood and midseason N fertilization are well understood in rice production, the effects and understanding of starter N applications to seedling rice are minimal and need to be investigated further. The objectives of this study were to evaluate the effects of starter N on seedling plant growth and grain yield, and to quantify the starter N recovery efficiency in a drill-seeded, delayed-flood rice system.

## Materials and Methods

### *Site Description and Cultural Practices*

Field studies were conducted at the Mississippi State University Delta Research and Extension Center, near Stoneville, MS, in 2007 and 2008, on Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) soil. Soil chemical properties included a pH of 8.0, organic matter content of 22 g kg<sup>-1</sup>, and a CEC of 42 cmol<sub>c</sub> kg<sup>-1</sup>. A factorial combination of two rice cultivars including a popularly grown semidwarf ‘Cocodrie’ (Linscombe et al., 2000), and a hybrid ‘XL723’ (RiceTec, Inc., Alvin, TX) and two levels of starter fertilizer {0 and 22 kg N ha<sup>-1</sup> as ammonium sulfate (AMS, 210 g N kg<sup>-1</sup>)} were arranged in a randomized complete block design, replicated four times, and produced in a

delayed-flood culture. Cocodrie was planted at 90 kg ha<sup>-1</sup> and XL723 was planted at 35 kg ha<sup>-1</sup>, which are common seeding rates for the inbred and F<sub>1</sub> hybrid, respectively. Experimental units measured 6.7 m in length and 1.6 m in width and were separated by 1.6 m alleys.

To contain <sup>15</sup>N-labeled AMS, four metal rings, each measuring approximately 0.7 m<sup>2</sup>, were evenly placed throughout the center of each experimental unit. Each ring was driven approximately 15 cm in to the ground to minimize movement of the labeled fertilizer in soil solution (Bufogle et al., 1997). For specified treatments, three of the four rings received <sup>15</sup>N-labeled AMS enriched at 10.1% atom at a rate of 22 kg N ha<sup>-1</sup> at the two-leaf rice growth stage. The fourth ring was used for grain yield (GY); therefore, it received non-labeled AMS at the same rate. All AMS was pre-weighed into scintillation vials. Immediately prior to application, the AMS was dissolved in 1 liter bottles containing 200 ml of water. The fertilizer mixture was broadcasted onto the soil surface when rice reached the two-leaf growth stage with a CO<sub>2</sub>-propelled, single nozzle spray wand. In both years, no rain events occurred within two days after application, and therefore plots were surface-irrigated to incorporate AMS into the soil. Plots treated with <sup>15</sup>N-labeled AMS at the two-leaf growth stage also received 146 kg N ha<sup>-1</sup> as granular urea (460 g N kg<sup>-1</sup>) at the five-leaf stage immediately prior to flooding. Plots not receiving two-leaf AMS application received 22 kg N ha<sup>-1</sup> as granular AMS and 146 kg N ha<sup>-1</sup> as urea at five-leaf rice prior to flooding. Four non-treated control plots were included for each cultivar to determine baseline native <sup>15</sup>N availability. To maintain proper flood conditions within the rings throughout the growing season, water levels

were routinely monitored and replenished by transferring water from the borrow ditches with a pail.

### *Data Collection*

Plant height was measured at five-leaf rice growth stage by randomly selecting individual plants from each treatment and averaging the measurements. Total aboveground biomass was hand harvested at pre-flood {PF (ring 1)}, panicle differentiation {PD (ring 2)}, and at 5% heading {HD (ring 3)} from each plot. All biomass was oven-dried at 60°C until a constant weight was obtained (48 to 72 hr) and weighed to determine total dry matter (TDM). Samples were then ground through a Wiley Mill. A dry combustion analyzer (Carlo Erba, Milan, Italy) coupled to an IsoPrime mass spectrometer (Micromass, Beverly, MA) was used for determination of the concentration of total plant N and atom percent  $^{15}\text{N}$  from 4 to 6 mg of sample. Total N uptake (TNU) was calculated by multiplying TDM and N concentration. The fourth ring was harvested with a hand-sickle and threshed with a stationary thresher (Almaco, Nevada, IA) at harvest maturity (150 to 180 g kg<sup>-1</sup> moisture content). Before statistical analysis, yields were standardized to 120 g kg<sup>-1</sup> moisture content.

Soil samples were collected from each plot prior to pre-flood N application and flood establishment and frozen at 4°C until analysis. Ammonium and NO<sub>3</sub><sup>-</sup> were extracted from 20 g of soil sample by adding 200 ml 1 N KCl. Soil extract NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were measured using an automated segmented flow analyzer (Perstorp Analytical Flow III Analyzer, Wilsonville, OR). Total soil atom percent  $^{15}\text{N}$  was determined using continuous flow isotope ratio mass spectrometry (CFIRMS).

### *Statistical Analysis*

Data collected prior to permanent flood establishment (plant height, TDM, TNU and N concentration) and GY were subjected to a test of main effects and analyzed by year using PROC MIXED in SAS. The model included the main effects of cultivar and starter N and interactions between the main effects. Replications were considered random. Least square means were calculated and mean separation ( $P \leq 0.05$ ) was produced using PDMIX800 in SAS (2003), which is a macro for converting mean separation output to letter groupings (Saxton, 1998).

Recovery of  $^{15}\text{N}$ -labeled AMS was also subjected to a test of main effects and analyzed separately by year using PROC MIXED in SAS. The model included main effects of cultivar and sample time and interactions of cultivar and sample time. Replications were considered random. Mean separation was conducted as previously described.

### **Results and Discussion**

There were no interactions between the main effects of cultivar and starter N for any of the measured parameters in 2007 and in 2008, thus the data presented are pooled over main effects. Starter N did not affect plant height or GY in 2007 or 2008 for either cultivar (Table 3.1). Nitrogen concentration was affected by starter N in both years (Table 3.1). In 2007 starter N resulted in an increase in N concentration; however, in 2008, N concentration was slightly lower where starter N was applied. Nitrogen concentration values ranged from 2.75% to 4.5% which is in the range reported by Dobermann and Fairhurst (2000). This provides justification in the decreased N



concentration observed in 2008 because greater TDM production resulted in the dilution of total plant N concentration. Total dry matter and TNU in 2008 were greater when starter N was applied. Approximately 180 kg ha<sup>-1</sup> of TDM was produced with a starter compared to 140 kg ha<sup>-1</sup> when none was applied. Additionally, 5.5 kg N ha<sup>-1</sup> was accumulated following a starter N application. Similar results for TDM and TNU were reported by Walker et al. (2008) for Cocodrie when grown on clay soils in Arkansas, Mississippi, and Missouri. In addition, studies conducted in Arkansas and Louisiana resulted in TNU of less than 10 kg N ha<sup>-1</sup> when measured at a similar growth stage (Bufogle et al., 1997).

The main effect of cultivar resulted in differences of all measured parameters except plant height in 2007 and TNU in 2008 (Table 3.1). In 2007, Cocodrie accumulated approximately 2 kg N ha<sup>-1</sup> more than XL723. In 2008, XL723 was 4 cm taller than Cocodrie at the five-leaf growth stage. Nitrogen concentration for XL723 was approximately 1% greater than Cocodrie in both years. Cocodrie produced 36 to 67% greater biomass than XL723 at the five-leaf growth stage. This is largely due to the fact that approximately three times the amount of seed is planted for Cocodrie compared to XL723. Hybrids are planted at low seeding rates due to their ability to produce a larger amount of reproductive tillers. Finally, XL723 produced approximately 25% greater GY. This yield difference is similar to that reported by Walker et al. (2008) for midsouthern United States rice production, and what has been reported in Asia (Surekha et al., 1999; Balasubramanian, 2002). Heterosis exhibited in F<sub>1</sub> plants contributes to greater root mass and yield components such as biomass, tillering, panicle length, and spikelet number per

panicle and are superior to the respective parent lines (Walker et al., 2008; Dobermann and Fairhurst, 2000; Li and Yuan, 2000).

Starter N fertilizer recovery was affected by an interaction between cultivar and sample time in 2007 (Table 3.2). When measured PF,  $^{15}\text{N}$ -AMS recovery was similar for both cultivars and averaged 0.75%. However, at PD and HD, XL723 recovered a greater percentage of  $^{15}\text{N}$ -AMS compared to Cocodrie. Recovery continually increased from PF to HD reaching maximum recoveries of 13.4% and 16.4% for Cocodrie and XL723, respectively. Greater hybrid root mass production as reported by others and observed by rice researchers in the midsouthern United States rice production region (Timothy Walker, personal communication) provides justification as to the greater recovery by XL723 in 2007 (Li and Yuan, 2000). The lack of  $^{15}\text{N}$ -AMS recovery at PF in 2007 further substantiates the lack of differences for plant height, TDM and TNU at the 5-leaf growth stage when starter N was applied.

In 2008,  $^{15}\text{N}$ -AMS recovery was only affected by sample time (Table 3.2). When pooled over cultivars, % $^{15}\text{N}$ -AMS recovery increased from approximately 3% at PF to a maximum of 8.3% at HD. These data show that approximately three times as much starter N was accumulated by the plants in 2008 relative to 2007. This provided some justification as to the greater TDM production and TNU measured in 2008 when starter N was applied.

When starter  $^{15}\text{N}$ -AMS recovery at the seedling rice stage was greatest, it was still minimal. The lack of starter N uptake by seedling rice can potentially be attributed to the nature of the drill-seeded system. Seed is drilled approximately 3.5 cm below the soil surface, and until a flood is established, rice roots typically extend downward into the

profile. Therefore, the mere proximity of the  $\text{NH}_4^+$  ion to the seedling rice roots may not allow for maximum N uptake. This coupled with the fact that a relatively small concentration of AMS was broadcasted to a high CEC soil surface could have impeded N uptake due to lack of ion movement. In many studies where starter fertilizer has increased height, biomass, or yield, the fertilizer source was banded in or near the row, which would allow for greater uptake (Vetsch and Randall, 2000; Osborne and Riedell, 2006).

Though a percentage of the starter AMS remains in a plant available form from the time of application until the cessation of N uptake, 2 to 4 kg N ha<sup>-1</sup> were not enough to affect grain production. Further soil analyses will provide insight as to how much of the AMS was available immediately after flooding. In theory, if a significant percentage of the AMS had converted to  $\text{NO}_3^-$ , then it would have been lost via denitrification.

### Conclusion

Starter AMS did not increase seedling plant height nor final grain yield. In 2008, greater TDM and TNU occurred when starter N was applied. This corresponded to greater %<sup>15</sup>N-AMS recovery when measured PF compared to 2007 when TDM and TNU was not impacted by starter N application. The most consistent differences stemmed from cultivars rather than starter N application. Relatively small amounts of starter AMS were absorbed by the plants throughout the duration of the growing season which provided insight to why only minimal affects were caused by starter AMS application. In addition to further investigation of the soil which will determine the relative concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  present in the soil at flooding, other studies should be

initiated to evaluate more effective application methods, rates, and sources to more consistently increase seedling growth.

Table 3.1 Individual treatment means, pooled means, and tests of main effects (cultivar and starter) for height, N conc., TDM, and TNU when measured at the five-leaf stage and grain yield in 2007 and 2008.

		2007					2008				
		Individual Treatment Means									
Cultivar	Starter kg N ha <sup>-1</sup>	Height cm	N conc. %	TDM	TNU kg ha <sup>-1</sup>	Yield	Height cm	N conc. %	TDM	TNU kg ha <sup>-1</sup>	Yield
Cocodrie	0	17.5	3.3	160	5.3	8954	19.5	2.8	173	4.8	9277
	22	16.8	4.0	164	6.4	8974	19.7	2.7	194	5.4	9328
XL723	0	17.2	4.2	107	4.5	11779	22.7	3.9	107	4.2	12101
	22	17.0	4.5	87	4.0	10986	24.6	3.5	164	5.6	11106
		Pooled Means									
Cocodrie		17.2	†3.6 b	162 a	5.9 a	8964 b	19.6 b	2.75 b	184 a	5.1	9303 b
XL723		17.1	4.4 a	97 b	4.2 b	11383 a	23.7 a	3.71 a	135 b	4.9	11604 <sup>a</sup>
	0	17.4	3.8 A	133	4.9	10367	21.1	3.35 A	140 B	4.5 b	10689
	22	16.9	4.2 B	125	5.2	9980	22.2	3.11 B	179 A	5.5 a	10217
		Tests of Fixed Effects P > f									
Cultivar		0.9166	0.0046	0.0008	0.0077	<0.0001	0.0037	<0.0001	0.0022	0.7459	0.0002
Starter		0.3513	0.0488	0.5557	0.6081	0.2650	0.3315	0.0449	0.0074	0.0465	0.2268
Cultivar*Starter		0.6395	0.3600	0.3898	0.1148	0.2436	0.4223	0.0557	0.1446	0.3811	0.1845

†Means within the same column and main effect followed by a different letter are significantly different at  $p \leq 0.05$ .

Table 3.2 Individual treatment means, pooled means, and tests of main effects (cultivar and sample time) for % <sup>15</sup>N recovery in 2007 and 2008.

Cultivar	Sample time	2007	2008
		% <sup>15</sup> N Recovery	
Individual Treatment Means			
XL723	PF	†0.5 e	3.0
	PD	10.1 c	4.7
	HD	16.4 a	8.8
Cocodrie	PF	1.0 e	2.5
	PD	8.0 d	4.6
	HD	13.4 b	8.8
Pooled Means			
XL723		9.0	5.5
Cocodrie		7.5	5.0
Tests of Fixed Effects			
		P > f	
Cultivar		0.0058	0.1219
Sample time		<0.0001	<0.0001
Cultivar*Sample time		0.0186	0.4324

†Means in the same column followed by a different letter are different at  $p \leq 0.05$ .

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