

IMPACT OF STARTER FERTILIZERS ON OPTIMUM RATE, TIMING AND EFFICIENCY  
OF NITROGEN FERTILIZER FOR GRAIN SORGHUM IN TEXAS

A Thesis

by

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## ABSTRACT

Nitrogen (N) management is critical for producing high yielding grain sorghum in Texas. Many producers utilize planter options for starter fertilizer application, including in furrow or 2x2 placements of N and phosphorus (P). N and P applied as starter fertilizers could affect optimum rate, timing and nitrogen use efficiency (NUE) of N fertilizer in grain sorghum. Advances in technology for remote sensing enable rapid acquisition of crop canopy spectral measurements. Analysis of spatial variability of crop canopy reflectance may enable site-specific nitrogen (N) management in grain sorghum.

Two field studies were established in Burleson County, Texas during 2016 and 2017 to impose contrasting N status in grain sorghum. The first study employed a single side-dress application of increasing N fertilizer rates (0, 112, 168, 224, 280 kg ha<sup>-1</sup>) for grain sorghum using three starter fertilizer applications. For the second study, timing (20, 42, 56, 65, 81 (2016) 32, 49, 66, 75, control (2017) days after planting) of side-dress N fertilizer application (168 kg N ha<sup>-1</sup>) was evaluated for grain sorghum under three starter fertilizer applications. Starter fertilizer treatments (sub-plots) included ammonium polyphosphate (11-37-0) applied at 0, 56 (in-furrow) or 168 (2x2) L ha<sup>-1</sup>. Spectral measurements (visible and NIR) of the sorghum canopy were made using ground-based at multiple dates during the growing season. Handheld sensors were also used to monitor N status throughout the season. Spectral indices and the handheld sensor values were used to evaluate and relate to crop biomass, grain yield and N content.

The optimal N rate in 2016 was 168 kg ha<sup>-1</sup>, there was no significance across increasing rates in 2017. Starter fertilizers did not affect the increasing rates. The optimal timing of N in 2016 was <56 days after planting in 2016, in 2017 there was not a significant difference with

delayed N application. Spectral measurements (visible and NIR) of the sorghum canopy were made using ground-based at multiple dates during the growing season. Handheld sensors were also used to monitor N status throughout the season. Spectral indices and the handheld sensor values were used to evaluate and relate to crop biomass, grain yield and N content.

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## NOMENCLATURE

N	Nitrogen
P	Phosphorus
NUE	Nitrogen Use Efficiency
NIE	Nitrogen Internal Efficiency
ANE	Agronomic Nitrogen Efficiency
NRE	Nitrogen Recovery Efficiency
PFP	Partial Factor Productivity
PNU	Plant Nitrogen Uptake
NDVI	Normalized Difference Vegetative Index
NIM	Nitrate Ion Meter
SPAD	Soil-Plant Analyses Development
DAP	Days After Planting
a.i.	Active Ingredient
n.s.	Not Significant

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1 CHAPTER I

2 INTRODUCTION

3  
4 Sorghum [*Sorghum bicolor* (L.) Moench] is a drought tolerant crop that is often grown  
5 under environmental conditions not suitable for many other crops (Moghaddam et al., 2007).  
6 Limited rainfall and dryland cropping systems provide opportunities for inclusion of grain  
7 sorghum in crop rotations in Texas. In 2017, Texas was the second largest producing state  
8 behind Kansas with 730, 000 thousand hectares of grain sorghum planted. In the United States,  
9 Kansas and Texas make up 75% of the total grain sorghum acres (USDA, 2017). Grain sorghum  
10 produced in Texas during 2016 was valued at \$388 million (USDA, 2017). Sorghum producers  
11 must optimize crop inputs to maximize yield and economic return. There are many inputs in  
12 producing high yielding sorghum with fertilizer being the single greatest input cost for  
13 production in Texas (Johnson, 2017).

14 Nitrogen (N) is the most limiting nutrients in producing high yielding grain sorghum. N  
15 plays a role in biological and physiological functions in plants, specifically in photosynthetic  
16 processes and protein building (Leghari et al., 2016). Consequently, N application in crop  
17 production is required due to the removal of N in grain or biomass and supply and retention of N  
18 by soil. When there is not an adequate amount of N, sorghum leaves may turn yellow and in  
19 severe cases premature leaf loss may occur (Vanderlip, 1993). The kernel weight, kernel size,  
20 and number of kernels are determined by the amount of N in the plant (Mahama et al., 2014;  
21 Vanderlip, 1993; Whitney, 1998). A grain yield of 6.3 Mg ha<sup>-1</sup> uses approximately 200 kg ha<sup>-1</sup>  
22 of N (Whitney, 1998). Therefore, it is necessary to fertilize the crop according to yield potential.  
23 The quantity of N applied is just one component of N management.

24           There are many different application methods for nitrogen fertilizer. Common practices  
25 in Texas include subsurface injection, surface dribble, broadcast, and broadcast incorporate.  
26 Nitrogen fertilizer can be split-applied (application at planting and application midseason) or  
27 applied all at once. Studies have demonstrated that a single application can have negative  
28 environmental effects (Cassman et al., 2002; Khosla et al., 2000; Randall and Hoelt, 1988;  
29 Tilman et al., 2002). This is due to N being a mobile nutrient in soil and susceptible to leaching  
30 below the root zone and subsequently reaching surface or ground water (Khosla et al., 2000;  
31 Whitney, 1998). Consequently, it is important to apply N in a method to reduce loss and  
32 increase fertilizer use efficiency (Cassman et al., 2002; Tilman et al., 2002). Numerous  
33 management strategies have emerged to increase the fertilizer efficiency and reduce loss.

34           Application of small amounts of fertilizer with or near the seed at planting is known as  
35 starter fertilizer application. According to Randall et al. (1985) the main objective to starter  
36 fertilizers is to increase fertilizer use efficiency. Various planter attachments are used to  
37 conveniently apply fertilizer at planting, reducing field traffic and saving time. Planter  
38 attachments offer two common options for applying starter fertilizer, in furrow (also known as  
39 pop up) or 2x2 (5 cm to the side of the seed trench and 0-5cm in depth). The placement of starter  
40 fertilizer is crucial in preventing seedling injury and providing economic benefits to the farm  
41 (Randall and Hoelt, 1988). The maximum rate of the starter is dependent on placement due to  
42 stand loss caused by a salt injury when starter placement is close to the seed (Raun et al., 1986).  
43 Potential benefits to starter fertilizer are an increase of early season growth, higher grain yield,  
44 and increased nitrogen use efficiency (Bermudez and Mallarino, 2002).

45           Split N applications can increase N use efficiency (NUE) and grain yields (Stecker et al.,  
46 1993). Split N applications also help provide an adequate amount of nitrogen during the grain fill

47 process in order to maximize yield. Growing point differentiation is when the plant initiates  
48 panicle development, about 30 days after emergence (Vanderlip, 1993). It is during this critical  
49 period that yield potential is established and when water or nutrient stress could reduce yield.  
50 Sorghum panicle initiation coincides with rapid nitrogen assimilation approximately 45 days  
51 after emergence. Most recommendations suggest having all N fertilizer applied by this time to  
52 maximize yield potential (Stichler et al., 1997). Starter fertilizer generally provides only a  
53 portion of crop N requirements, requiring subsequent N applications to meet total crop demand.  
54 How starter fertilizers influence optimum rate and timing of N fertilizer in sorghum is uncertain.

55 Determining the optimum N rate is essential for efficient N use in the plant and for proper  
56 plant development (Buah et al., 2012; Fageria and Baligar, 2005b; Mahama et al., 2014; Mahama  
57 et al., 2016; Maman et al., 2017). Studies have shown that increased N rates result in sorghum  
58 reaching flowering quicker (Mahama et al., 2014; Mahama et al., 2016). Sorghum height and  
59 biomass are also positively affected by increasing N application rates (Buah et al., 2012; Maman  
60 et al., 2017). Inadequate N application rate is detrimental to grain yield, resulting in reduced  
61 grain fill or small kernel size (Mahama et al., 2014). Current fertilization recommendations for  
62 grain sorghum in Texas are yield-based, with 2 kg of N for 100 kilograms per hectare of grain  
63 (Stichler et al., 1997). Excess N application can negatively affect soil and water quality and  
64 reduce NUE, meaning growers spend more than necessary to meet crop needs for N (Cassman et  
65 al., 2002).

66 In addition to simply N in starter fertilizers, applying N and phosphorus (P) fertilizer at  
67 planting could affect the optimum rate and timing of N side dress fertilizer. Previous studies have  
68 shown improved growth rate at the beginning of the season when N and P starter fertilizer is  
69 used instead of N only starter (Gordon and Whitney, 1995; Touchton and Karim, 1986).

70 Improved early season growth and vigor could impact yield potential and optimum N  
71 requirements. Moreover, early season nutrient demands of grain sorghum could be satisfied by  
72 starter fertilizer applications and potentially expand the optimum window for N side dress  
73 applications. N side dress timing should be further evaluated for fertilizer programs utilizing  
74 common starter fertilizer practices.

75         Optimizing N fertilizer programs for contrasting starter fertilizer practices is expected to  
76 contribute to greater NUE in grain sorghum. Improving NUE will provide environmental  
77 benefits for Texas and economic benefits for producers. Tilman et al. (2002) stated improving  
78 NUE in cereal grains can lead to higher yields and quality while reducing N losses. Cassman et  
79 al. (2002) discusses how applied N that is not taken up by the crop is prone to volatilization,  
80 denitrification, and leaching, which can lead to economic and agronomic farm losses and  
81 environmental hazards over time. Low NUE can decrease water quality, aquatic habitats, and  
82 increase purification costs for drinking water (Cassman et al., 2002; Tilman et al., 2002). This  
83 can help producers get a higher economic benefit from their fertilizer.

84         Many measurements and calculations have been used to describe NUE of grain crops and  
85 cropping systems (Fageria and Baligar, 2005a). NUE calculations are designed to provide  
86 estimates of output (kg of grain) per unit of input (kg of N). Calculations are commonly based on  
87 the difference method, where reference plots with no added N are compared to plots receiving N  
88 fertilizer. NUE and agronomic N efficiency (ANE) are synonymous (in most publications) and  
89 calculated as shown below. Additional terms used related to NUE include: partial factor  
90 productivity (PFP), nitrogen recovery efficiency (NRE), nitrogen internal efficiency (NIE) and  
91 plant N uptake (PNU);

$$ANE = \frac{(Y_n - Y_0)}{N_r}$$

$$PFP = \frac{Y_n}{N_r}$$

$$NRE = \frac{(PNU_n - PNU_0)}{N_r}$$

$$NIE = \frac{(Y_n - Y_0)}{(PNU_n - PNU_0)}$$

92           Where  $Y_n$  is grain yield ( $\text{kg ha}^{-1}$ ) for plots receiving fertilizer N,  $Y_0$  is grain yield ( $\text{kg ha}^{-1}$ )  
93 for plots without fertilizer N,  $N_r$  is the applied N fertilizer rate ( $\text{kg ha}^{-1}$  of N),  $PNU_n$  is plant N  
94 uptake ( $\text{kg ha}^{-1}$ ) of N fertilized plot,  $PNU_0$  is plant N uptake ( $\text{kg ha}^{-1}$ ) of unfertilized plot. NUE  
95 measurements are essential for evaluation of contrasting fertilizer and N management practices.  
96 A wide range of NUE for contrasting rate, timing and placement of N in corn and other grain  
97 crops have been reported for some regions of the U.S. (Cassman et al., 2002; Fageria and  
98 Baligar, 2005a). NUE for corn has been reported between 19 and 42 ( $\text{kg grain kg}^{-1}$  N) depending  
99 on management practices evaluated (Attia et al., 2015; Ciampitti and Vyn, 2014; Jaynes, 2015;  
100 Kovács et al., 2015a). Information on NUE for sorghum is limited. Understanding how common  
101 fertilizer practices interact with N management to affect NUE is urgently needed for sorghum.

102           The availability of crop sensors, unmanned aerial systems (UAS), satellite imagery and  
103 data management systems is enabling farmers to collect and analyze spatial reflectance data that  
104 may be used to improve crop N management (Clay, 2011; Graeff and Claupein, 2003; Pierce and  
105 Nowak, 1999; Thorp et al., 2015; Zhang and Kovacs, 2012). The potential ability spectral  
106 sensors have to detect N stress and allow farmers to alter management of fertilizer inputs could  
107 further contribute to increases in NUE in grain sorghum (Graeff and Claupein, 2003; Singh et al.,  
108 2017; Zhao et al., 2005). Yet the ability of spectral sensors to detect N stress in sorghum and at

109 developmental stages that enable corrective action is uncertain. Furthermore, deficiencies in total  
110 N supply to grain sorghum could be masked by early season fertilizer practices, including starter  
111 fertilizer practices.

112           Grain sorghum yield and crop development are affected by nutrient management  
113 practices. Minimal research has been done in Texas to measure the grain and biomass yield, and  
114 NUE for grain sorghum response to starter fertilizers and N management. This study serves to  
115 improve our understanding of three main objectives. 1) Measure and compare grain sorghum  
116 yield in response to nitrogen fertilizer rates for sorghum with contrasting starter fertilizer  
117 practices. Determine NUE for contrasting starter practices with increasing fertilizer rates. 2)  
118 Measure and compare grain sorghum yield in response to nitrogen fertilizer timing for sorghum  
119 with contrasting starter fertilizer practices. Determine NUE for contrasting starter practices with  
120 increasing delay of side-dress N fertilizer application. 3) Evaluate sorghum leaf and canopy level  
121 reflectance under contrasting starter fertilizer and N management practices. Relate leaf and  
122 canopy reflectance to stem nitrate-N concentration, whole plant tissue N concentration, crop  
123 biomass and grain yield.



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## CHAPTER II

### STARTER FERTILIZERS AND OPTIMUM NITROGEN RATE IN GRAIN SORGHUM

#### **Introduction**

Starter fertilizer applications involve injecting small amounts of fertilizer with or near the seed at planting. While starter fertilizers are common practice in many crops and production regions, limited information is available for grain sorghum. There are many options for placement, rate, and nutrient combinations in starter fertilizers. Starter fertilizer applications generally apply small amounts of N and P and can affect crop development and grain yield potential (Bermudez and Mallarino, 2002; Gordon and Whitney, 1995; Reeves et al., 1990). Essentially, starter fertilizers that contain N result in split application of N fertilizer. Split application of N fertilizer can increase NUE compared to single applications (Bermudez and Mallarino, 2002; Gordon and Whitney, 1995; Niehues et al., 2004; Scharf, 1999; Soroka, 2016; Touchton and Karim, 1986). With the potential to alter NUE and grain sorghum yield potential, optimum N fertilizer rates may be affected by starter fertilizer practices.

Placement of the starter fertilizer can affect crop growth and yield (Randall and Hoefft, 1988). There are many different placement options, including in-furrow, 2x2 (5cm to the side of the seed and 0-5cm below the soil surface), and broadcasted (Randall et al., 1985). In-furrow placement can increase seedling growth due to increased availability of nutrients in close proximity to the seed (Niehues et al., 2004). However, placing too much fertilizer too close to the seed can cause negative effects, such as stand reduction and slower emergence, due to salt injury (Raun et al., 1986). Niehues et al. (2004) showed that 2x2 placement decreases the potential for fertilizer salt injury. Physical separation of fertilizer bands and seed reduces injury potential yet

147 is readily available to seedlings to promote efficient uptake of nutrients and rapid growth.  
148 Reeves et al. (1990) found placement of the starter solutions in-furrow or 2x2 both increased  
149 grain sorghum growth during the beginning of the season. Yet, the rate of the starter fertilizer  
150 and associated N content can impact the seedling growth and the crop yield (Scharf, 1999).

151         The impact of N on sorghum growth and development is well documented. A study in  
152 Kansas showed increasing N rates applied by hand 10-14 days after emergence results in a  
153 shorter time to flowering (Mahama et al., 2016). Mahama et al. (2016) found there was an  
154 increase of 4-8 days to flowering when comparing 45 kg N ha<sup>-1</sup> applied to 90 kg N ha<sup>-1</sup> applied  
155 to the sorghum. Mahama et al. (2014) also showed a 3-5 day reduction to flowering in multiple  
156 hybrids with N applied compared to no N applied. This indicates the sorghum development rates  
157 are greater as rates of N fertilizer increase. In addition to earlier flowering, sorghum biomass and  
158 plant height are affected by N application rates (Buah et al., 2012; Mahama et al., 2014; Mahama  
159 et al., 2016; Maman et al., 2017). Maman et al. (2017) observed an increase in biomass by 31  
160 percent with N applied compared to no N. In Kansas, Mahama et al. (2014) found an increase in  
161 plant height when 45 kg N ha<sup>-1</sup> was added, but there was not a difference in plant height with  
162 more than 45 kg N ha<sup>-1</sup>. Therefore, N found in starter fertilizers and potential adjustments to  
163 optimum N fertilizer rates could affect growth and development in sorghum.

164         In addition to growth and development, grain yield and associated yield components can  
165 be altered with increasing N application rates. Mahama et al. (2016) reported an increase of  
166 kernels per head, kernel weight, and yield when increasing N rates were applied. Buah et al.  
167 (2012) found in Uganda with N depleted soil there was a significant increase in yield up to 80 kg  
168 N ha<sup>-1</sup>. A long term study in Kansas (Schlegel and Bond, 2016) showed that N alone can increase  
169 yields when 180 kg N ha<sup>-1</sup> is applied on a soil with high K content. Differences in optimum N

170 rates for contrasting studies may reflect differences in yield potential due to variation of  
171 environmental conditions and management practices. Likewise, differences in yield potential due  
172 to starter fertilizers could influence optimum N fertilizer rates.

173 Grain sorghum yield and crop development are affected by nutrient management  
174 practices. Minimal research has been done in Texas to measure the grain and biomass yield, and  
175 NUE for grain sorghum response to starter fertilizers and N management. The objectives of this  
176 study was to measure grain sorghum yield in response to nitrogen fertilizer rates for sorghum  
177 with contrasting starter fertilizer practices. To relate grain sorghum yield to nitrogen fertilizer  
178 rates for three starter fertilizer practices (no starter fertilizer, popup and 2x2) using linear and  
179 nonlinear regression. Determine NUE for contrasting starter practices with increasing fertilizer  
180 rates.

## 181 **Materials and Methods**

182 A two-year study was conducted in Burleson County, TX at the Texas A&M AgriLife  
183 Research farm (30.543297, -96.435316) to evaluate the impact of starter fertilizer on optimum  
184 rate of N fertilizer in grain sorghum under non-irrigated conditions. The study site is  
185 predominately Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic  
186 Haplustepts) and Ships clay (very-fine, mixed, active, thermic Chromic Hapluderts) under  
187 conventional tillage using raised beds. Corn was the previous crop both years.

188 The study was arranged in a split plot design with four replications each of five main  
189 plots and three subplot fertilizer treatments for grain sorghum production during 2016 and 2017.  
190 Five nitrogen rates (0, 112, 168, 224, 280 kg N ha<sup>-1</sup>) were the main plot and three starter fertilizer  
191 practices (in furrow, 2x2, none) were the sub-plot. Grain sorghum was planted on April 6, 2016  
192 and March 22, 2017, both years using Dekalb DKS 51-01. A four-row John Deere 1705 planter

193 was used to seed 160,550 seeds ha<sup>-1</sup> on 76.2 cm spacing. The treatments of the starter solutions  
194 were liquid ammonium polyphosphate (11-37-0) applied at 1) In-Furrow - 56 L ha<sup>-1</sup> applied in  
195 the seed furrow 2) 2x2 - 168 L ha<sup>-1</sup> placed 5 cm to the side of the seed trench and up to a 5cm in  
196 depth and 3) No Starter - check. Subplots were four rows wide and 20.1 (2016) and 30.5 (2017)  
197 m in length. N rate treatments were applied at pre-boot (2016 delayed due to wet weather) and 6-  
198 leaf stage (2017) using a Scotts Turf Builder Classic Drop Spreader to band granular N fertilizer  
199 between rows. Granular urea (46-0-0) was applied to obtain the total N rates of: 1) 112kg N ha<sup>-1</sup>  
200 2) 168 kg N ha<sup>-1</sup> 3) 224 kg N ha<sup>-1</sup> 4) 280 kg N ha<sup>-1</sup> (adjusted for N applied through starter  
201 practice).

202 Pesticide management strategies followed Agrilife recommendations. In 2016 a pre-  
203 emergence application consisted of Roundup WeatherMax at 2.8 L ha<sup>-1</sup> (a.i. glyphosate 48.8%),  
204 Dual II Magnum at 1.4 L ha<sup>-1</sup> (a.i. s-metachlor 82.4%), and AAtrex 4L at 2.8 L ha<sup>-1</sup> (a.i. atrazine  
205 42.6%). Cultivation was used pre-boot to control weeds during 2016. During 2016, Prevathon at  
206 1 L ha<sup>-1</sup> (a.i. chlorantraniliprole 5%) was used to control head insects (*H. zea*, *S. frugiperda*, *Nola*  
207 *sorghiella*, *Nezera viridula*, *Oebalus pugnax*). In 2017 a pre-emergence application of Outlook  
208 at 1 L ha<sup>-1</sup> (a.i. dimethenamid-P 63.9%) and Roundup WeatherMax at 2.8 L ha<sup>-1</sup> (a.i. glyphosate  
209 48.8%) was applied. Cultivation practices occurred during growth stage 3 to remove grass and  
210 broadleaf weeds growing in between the rows in 2017. An in-season application of AAtrex 4L at  
211 3.5 L ha<sup>-1</sup> (a.i. atrazine 42.6%) and Rifle at .58 L ha<sup>-1</sup> (dicamba 48.2%) were also be applied at  
212 growth stage 4 to suppress broadleaf weeds. An application of Tombstone at 70 mL ha<sup>-1</sup> (a.i.  
213 cyfluthrin 24.74%) was applied for midge (*Stenodiplosis sorghicola*) control on May 31. An  
214 application of Prevathon at 1 L ha<sup>-1</sup> (a.i. chlorantraniliprole 5%) and Silencer at 0.21 L ha<sup>-1</sup> (a.i.

215 lambda-cyhalothrin 12.7%) insecticides were applied growth stage 7 (6/20/2017) for headworm  
216 and stinkbug control (*H. zea*, *S. frugiperda*, *Nola sorghiella*, *Nezera viridula*, *Oebalus pugnax*).

217 Grain yield, moisture, and test weight were measured from the center two rows of each  
218 plot using a John Deere 3300 plot combine with a Harvest Master (HM800) on-board weigh  
219 system. Grain yield was adjusted to 14% moisture. In 2016 there were skips, due to poor  
220 establishment caused by ponded water after planting. The skips were measured and deducted  
221 from plot lengths to correct actual combine harvested area. In addition, all plants from a 3 m  
222 section of the first row of each plot was harvested (cut at the soil level by hand), separated into  
223 grain and aboveground biomass to measure dry matter and nitrogen content. Panicle weight,  
224 grain weight, test weight, moisture, and seed size were measured to calculate grain yield. The  
225 grain was milled (2 mm) and submitted for measurement of total nitrogen content. Sorghum  
226 aboveground biomass was weighed, chopped in the field using a forage chopper (Case 700). A  
227 subsample was weighed and dried (65C) using a forced air oven for 36 hrs or until moisture loss  
228 is minimal. Dry matter and moisture content was used to calculate dry matter yield for  
229 aboveground biomass. Dried subsamples were ground using a Wiley Mill 4 (2mm) and sent to  
230 the laboratory for N analysis. The tissue and grain samples collected at hand harvest were  
231 submitted to the Texas A&M Forage, Soil, Water Laboratory (College Station, TX) to measure  
232 total nitrogen in biomass and grain samples. Total N (plant tissue and grain) was determined by  
233 high-temperature combustion (McGeehan and Naylor, 1988).

234 Soil samples were collected at the beginning of the growing seasons to determine soil  
235 concentration of nitrate-N and extractable nutrients. Soil cores were collected using a GSRPS  
236 Giddings Machine Company soil probe. Soil was sampled 60 cm in depth and divided into 15 cm  
237 segments. The samples were sent to the Texas A&M Forage, Soil, Water Testing Laboratory for

238 analysis. Mehlich III was used to extract phosphorus, potassium, calcium, magnesium, sodium,  
239 and sulfur and concentration measured by inductively coupled plasma (ICP) and atomic emission  
240 spectroscopy (Mehlich, 1984). A hydrogen selective electrode was used to determine soil pH in a  
241 1:2, soil: deionized water extract. A conductivity probe was used to find soil electrical  
242 conductivity with the same soil: deionized water ratio as the soil pH (Schofield and Taylor,  
243 1955). Nitrate nitrogen was extracted from the soil with 1 N KCL solution(Keeney and Nelson,  
244 1982).

245 NUE was calculated five ways: agronomic N efficiency (ANE), partial factor  
246 productivity (PFP), N recovery efficiency (NRE), N internal efficiency (NIE) and plant N uptake  
247 (PNU).

$$ANE = \frac{(Y_n - Y_0)}{N_r}$$

$$PFP = \frac{Y_n}{N_r}$$

$$NRE = \frac{(PNU_n - PNU_0)}{N_r}$$

$$NIE = \frac{(Y_n - Y_0)}{(PNU_n - PNU_0)}$$

$$PNU = \text{biomass N uptake} + \text{grain N uptake}$$

248 Where  $Y_n$  is grain yield ( $\text{kg ha}^{-1}$ ) for plots receiving fertilizer N,  $Y_0$  is grain yield ( $\text{kg ha}^{-1}$ ) for  
249 plots without fertilizer N,  $N_r$  is the applied N fertilizer rate ( $\text{kg ha}^{-1}$  of N),  $PNU_n$  is plant N uptake  
250 ( $\text{kg ha}^{-1}$ ) of N fertilized plot,  $PNU_0$  is plant N uptake ( $\text{kg ha}^{-1}$ ) of unfertilized plot.

251 Statistical analyses was performed using SAS 9.4 (Cary, NC). The PROC GLM  
252 procedure was used to analyze variables of grain yield, NUE, biomass yield, grain and biomass

253 N as a split-plot design with fertilizer rate as the main plot and starter fertilizer as the subplot  
 254 with years analyzed independently. When appropriate, means were compared using Fisher's  
 255 L.S.D.

## 256 **Results and Discussion**

### 257 *Soil Analysis*

258 In both years of the study the mean P levels were optimum, which indicates a response to  
 259 P fertilization may or may not occur (Table 1). Potassium (K), Calcium (Ca), and Magnesium  
 260 (Mg) was optimum or above optimum. NO<sub>3</sub>-N in 2016 was higher in the top 15cm than 2017. In  
 261 2017 10.3 ppm of NO<sub>3</sub>-N was found in the top 0-60cm.

263 **Table 1 Soil Analysis from the Top 15cm**

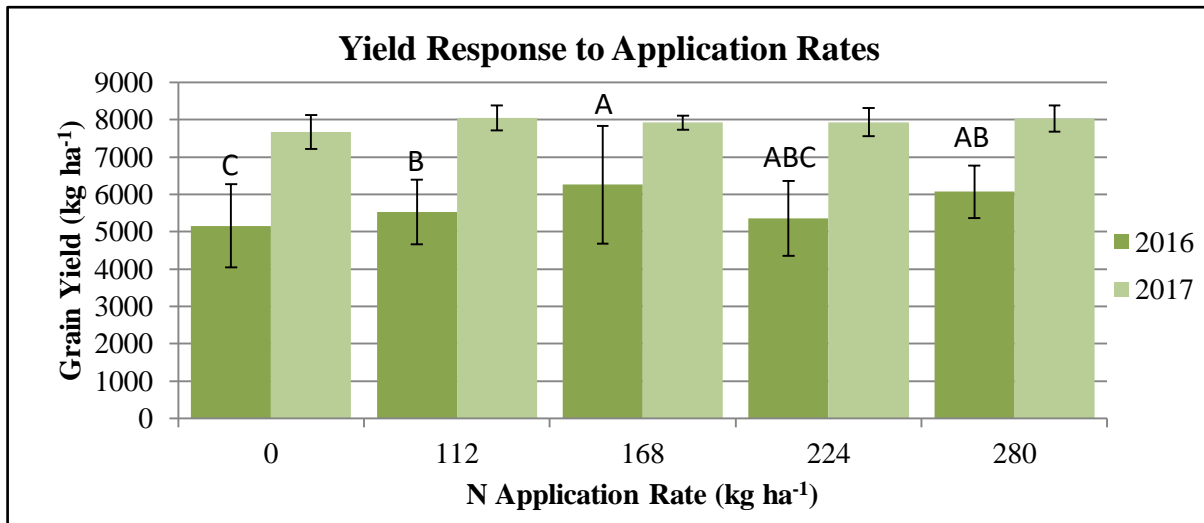
Year	pH	Conductivity Umhos cm <sup>-1</sup>	NO <sub>3</sub> -N ppm	P ppm	K ppm	Ca ppm	Mg ppm	S ppm	Na ppm
2016	8	230	8.9	51	245	5672	210	11	23
2017	8	369	4	52	209	4999	178	9	8

### 266 *Grain and Biomass Yield*

267 Analysis of grain yield revealed that main effects of N rate and starter practices were  
 268 significant in one year of the two-year study. Nitrogen fertilizer rate contributed significantly to  
 269 ( $p < 0.01$ ) increase in grain yield during 2016. In 2016, mean grain yields ranged from 5,156 to  
 270 6,258 kg ha<sup>-1</sup> with increasing rates of N fertilizer applied (Figure 1). Application rates of 168 to  
 271 280 kg N ha<sup>-1</sup> were associated with an increase in grain yield as much as 21% compared to 0 kg  
 272 N ha<sup>-1</sup>. Grain yield did not increase above the application rate of 168 kg N ha<sup>-1</sup> during 2016. In  
 273 2017, grain yields were greater than 2016, however, there was not a significant difference in

274 grain yield due to N application rate (Figure 1). Mean grain yield ranged from 7,671 to 8,042 kg  
275 ha<sup>-1</sup> during 2017.

276 Despite lack of grain yield response during 2017, 2016 results were similar to other  
277 reports for grain sorghum response to N fertilizer. In Kansas, Schlegel and Bond (2016) found N  
278 applications up to 180 kg N ha<sup>-1</sup> were associated with increased yields. Mahama et al. (2016),  
279 found an optimum N rate of 90 kg N ha<sup>-1</sup> in a fallow cropping system gave the best economic  
280 returns. Grain yields, where no N fertilizer was applied, were 5,156 and 7,671 kg ha<sup>-1</sup> in 2016  
281 and 2017 in the current study. Previous studies found much lower levels of grain production  
282 when no N fertilizer was applied (Mahama et al., 2014; Mahama et al., 2016; Maman et al.,  
283 2017). This suggests that antecedent soil nitrogen contributed significantly to grain yield.  
284



**Figure 1 Yield Response to Application Rates.** Average grain yield response to increasing N rates in 2016 and 2017. The different letters indicate significant differences with in N rates at P < 0.05. Error bars represent one standard deviation.



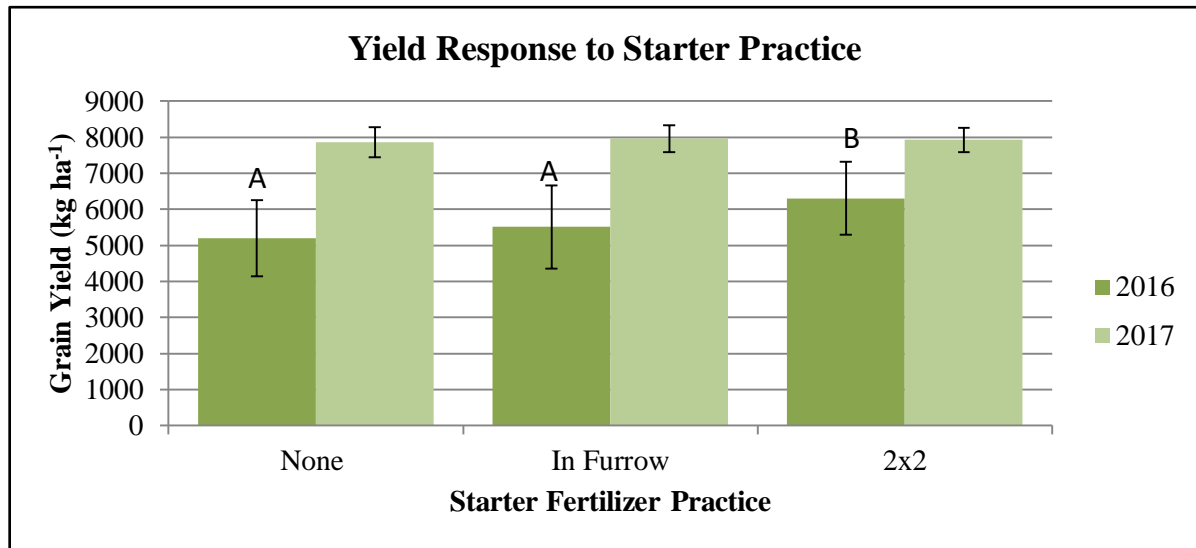
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287           Antecedent soil nitrogen comprises residual soil nitrate-N and mineralization of organic  
288 N during the growing season. Residual soil nitrate-N averaged 19 kg ha<sup>-1</sup> in the top 15 cm  
289 (2016), 8 kg ha<sup>-1</sup> in the top 15 cm (2017) and 46 kg ha<sup>-1</sup> in the top 0-60 cm (2017). Crop residue  
290 from previous corn crops may have contributed to plant available N pools during the subsequent  
291 crop year (Blanco-Canqui and Lal, 2009; Dolan et al., 2006; Mullen et al., 2010; Salinas-Garcia  
292 et al., 2001). Mullen et al. (2010), found N content of 0.69% in corn cobs and 0.79% N content  
293 in corn stover when looking at potential removal of residues for biooil and biochar production.  
294 Salinas-Garcia et al. (2001), found the potential mineralizable N to be 4 kg ha<sup>-1</sup> day<sup>-1</sup> from corn  
295 residue in a conventional tilled system in Mexico. In a corn stover removal study, Blanco-Canqui  
296 and Lal (2009) , found a 25-26% decrease in total soil N to a depth of 10 cm when they removed  
297 all stover, but the decrease was soil specific with silty loams texture class.

298           Previous year corn yields ranged from 11,300 kg ha<sup>-1</sup> to 12,550 kg ha<sup>-1</sup> at the current  
299 study site. Higher N fertilizer rates used to obtain greater corn yields can result in greater organic  
300 carbon and N returned to soil (Liebig et al., 2002). Burgess et al. (2002) found that 70 to 80% of  
301 N in corn residues was released over a 2-year period under field conditions in Canada. It is likely  
302 the previous year's corn residue provided a source of mineralizable N in the current grain  
303 sorghum study.

304           Variation in grain yield was observed for contrasting starter fertilizer practices in 2016.  
305 Starter fertilizer practice did not affect (p>0.05) grain yields in 2017. Grain yield ranged from  
306 5,000 to 6,000 kg ha<sup>-1</sup> in 2016 and from 7,900 to 8,000 kg ha<sup>-1</sup> in 2017 (Figure 2). In 2016,  
307 applying starter fertilizer as a 2x2 increased grain yield by 12.52% compared to the in-furrow

308 and 14.2% compared to treatments with no starter fertilizer applied. In-furrow starter fertilizer  
 309 application did not increase grain yield compared to treatments without starter fertilizer. While  
 310 2x2 starter fertilizer applications did increase grain yield, it also provides greater amounts of N



311 and P compared to in-furrow applications. Scharf (1999) found comparable results in a corn N  
 312 management system with the largest increase in yield being 1632 kg ha<sup>-1</sup> with a 2x2 starter of 11-  
 313 44-44 compared to a no starter treatment.

314

**Figure 2 Yield Response to Starter Practice.** Average grain yield response to starter practices in 2016 and 2017. The different letters indicate significant differences with in starter practices at P < 0.05. Error bars represent one standard deviation.

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317 Biomass production was not affected by N treatments in either year. Despite differences  
 318 in grain yield in 2016 there was no effect on biomass yield. The average biomass yield in 2016  
 319 and 2017 was 8,130 kg ha<sup>-1</sup> and 10,532 kg ha<sup>-1</sup>. Powell and Hons (1992) found contrary results,  
 320 where there was an increase in biomass production up to 112 kg N ha<sup>-1</sup> applied in their  
 321 treatments. There was a positive significant increase in biomass with 224 kg N ha<sup>-1</sup> applied in

322 two of the four years with a conventional grain sorghum cultivar (ATx623 x RTx430) and  
323 intermediate type sorghum cultivars (ATx423 x RIO, ATx423 x HEGARI). Hons et al. (1986)  
324 found an increase in sorghum biomass production with increase N rates in a study comparing  
325 genotype response to N. All 3 genotypes (high energy sorghum, intermediate type sorghum,  
326 grain sorghum) responded positively to application rates up to the highest rate of 168 kg N ha<sup>-1</sup>.

### 327 *Plant Nitrogen Concentration and Uptake*

328 Grain and aboveground biomass was sampled and analyzed separately for N  
329 concentration where mass of N in each treatment was determined. Grain N concentration and  
330 total N uptake was affected by N rate in both years (table 1). Grain N concentration ranged from  
331 12.1 to 15.7 g kg<sup>-1</sup> in 2016 and 11.2 to 13.3 g kg<sup>-1</sup> in 2017. Increasing N fertilizer rates increased  
332 grain N concentration (p<0.003) 22% in 2016 and 16% in 2017 comparing the control N rate to  
333 the highest N rate. Total N uptake by grain ranged from 57.4 to 88.4 kg ha<sup>-1</sup> in 2016 and 74.4 to  
334 93.1 in 2017. Starter fertilizer practice did not affect N content of grain in either year and  
335 resulted in some variation in total N uptake by grain in one of two years. Hons et al. (1986)  
336 found similar results where the N concentration and PNU increased with the increasing N  
337 applications.

338 Aboveground biomass (excluding grain) N concentration was also affected by N fertilizer  
339 rates. Increasing N fertilizer rates increased biomass N concentration in 2016 and 2017 (Table 2).  
340 Aboveground biomass tissue N concentration ranged from 7.2 to 10.0 g kg<sup>-1</sup>. Biomass N  
341 concentration was increased 25% comparing 0 to 280 kg N ha<sup>-1</sup> in 2017. Hons et al. (1986) found  
342 a larger increase of N concentration when comparing 0 to 84 kg N ha<sup>-1</sup> and 168 kg N ha<sup>-1</sup> with a  
343 24-75% increase. However, total N uptake in aboveground biomass was not affected by N rate in  
344 either year. Variation in aboveground biomass yield likely overshadowed variation in biomass N

345 concertation due to N rates. Starter fertilizer practices resulted in variation in biomass N  
 346 concentration in 1 of 2 years and did not affect total N uptake.

347 Total plant N uptake (PNU) was affected by increasing N rates in 1 of the 2 years. PNU  
 348 was significantly ( $p < .05$ ) increased, in 2016, with N rates up to 224 kg N ha<sup>-1</sup>. There was a 43.6  
 349 kg N ha<sup>-1</sup> increase from the control (no N applied pooled across starter treatments) and the 224  
 350 kg N ha<sup>-1</sup> rate. Similar PNU was reported by Ciampitti and Prasad (2016) with mean PNU of 138  
 351 kg N ha<sup>-1</sup> from years 1965 - 2014 over 13 studies. The review paper found the studies conducted  
 352 for 2001-2014 had lower mean PNU values of 60-80 kg N ha<sup>-1</sup>.

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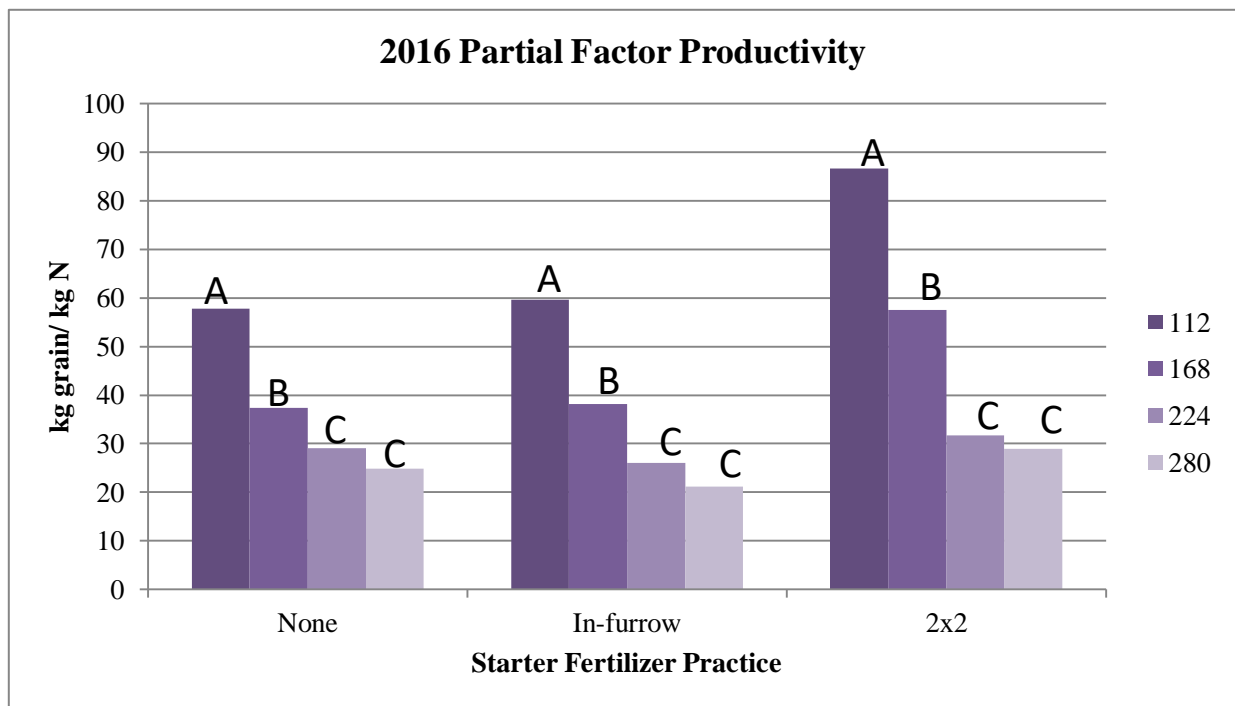
354 **Table 2 Plant Nitrogen Concentration and Nitrogen Uptake with Contrasting Application Rates.** Average N  
 355 concentration and N uptake of starter practices and increasing N rates for grain and biomass in 2016 and 2017.  
 356 L.S.D. was used to compare means.

	N Concentration (g kg <sup>-1</sup> )		N Uptake (kg ha <sup>-1</sup> )		
	Biomass	Grain	Biomass	Grain	PNU
<b>Starter</b>	<b>2016</b>				
None	9.3	14.4	76.4	80.0	156.4
In-Furrow	8.4	14.0	69.8	67.7	137.5
2x2	9.4	14.1	74.6	76.9	151.5
<i>Pr &gt; F</i>	0.022	n.s.	n.s.	0.012	n.s.
<b>N-Rate</b>					
0	8.1	12.1	60.1	57.4	117.5
112	8.5	13.4	76.1	70.7	146.8
168	9.2	14.6	76.0	81.8	157.8
224	9.5	14.8	85.0	76.1	161.1
280	10.0	15.7	70.7	88.4	159.1
<i>Pr &gt; F</i>	0.003	<0.0001	n.s.	<0.0001	0.0095
<b>Starter</b>	<b>2017</b>				
None	8.4	12.4	88.6	84.8	169.5
In-Furrow	8.8	12.8	88.6	88.6	175.1
2x2	8.8	12.9	98.5	88.2	182.4
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	n.s.	n.s.
<b>N-Rate</b>					
0	7.2	11.2	71.4	74.4	140.5
112	8.7	12.9	88.4	90.1	175.5
168	8.8	13.1	95.7	90.2	179.0
224	9.2	13.0	106.4	90.1	197.3

280	9.6	13.3	98.1	93.1	190.4
<i>Pr &gt; F</i>	0.0005	<0.0001	n.s.	0.0005	n.s.
n.s. = not significant (>0.05), PNU (total plant N uptake)					

357 *Nitrogen Use Efficiency*

358 Mean Partial factor productivity (PFP) was 33.1 kg kg<sup>-1</sup> in 2016 and 46.1 kg kg<sup>-1</sup> in 2017  
 359 (Table 2). Mean PFP was affected by N rate in both years of the study and was affected by starter  
 360 fertilizer in one of two years. Mean PFP declined (p < 0.0001) 25 and 34% with N rates  
 361 increasing from 112 to 168 kg N ha<sup>-1</sup> in 2016 and 2017. Abunyewa et al. (2017) found similar  
 362 results for PFP in sorghum, with a 23% decrease in PFP when N rates increased from 100 to 150  
 363 kg N ha<sup>-1</sup>. In 2016, there was an interaction between starter practices and N rates for PFP  
 364 (Figure 3). Mean PFP increased (p < 0.05) for in-furrow and 2x2 compared to grain sorghum



**Figure 3 2016 Partial Factor Productivity.** Average PFP of starter practices and increasing N rates in 2016. The different letters indicate significant differences of N rates with in each starter practice at P < 0.05. Legend units are kg N ha<sup>-1</sup>.

365 without starter fertilizer applied.

366 Mean NRE ranged from 18.1 to 26.4% across all years and fertilizer treatments (Table 3).  
367 NRE did not differ among starter practices and N fertilizer rates in 2016 or 2017. The NRE mean  
368 values were lower than when compared to other studies. In a N management study with corn  
369 Kovács et al. (2015b) found higher NRE values of 43-81% recovery. In two of the three years of  
370 the study 90 kg N ha<sup>-1</sup> had the highest NRE and it decreased with increasing rates up to 202 kg N  
371 ha<sup>-1</sup> (Kovács et al., 2015b). Gagnon and Ziadi (2010) conducted a corn N management study  
372 comparing three different N sources (urea ammonium nitrate [UAN], calcium ammonium  
373 nitrate [CAN], aqua ammonia [AA]) and found a wide range NRE values from 19-75% recovery.  
374 UAN had the highest recovery of 75% with 100 kg N ha<sup>-1</sup> as the N application rate. While, AA  
375 had the lowest NRE at 19% with a N rate of 200 kg ha<sup>-1</sup>.

376 Mean nitrogen internal efficiency (NIE) ranged from 10.8 to 53.2 kg kg<sup>-1</sup> across both  
377 years and all fertilizer treatments (Table 2). Mean NIE did not differ among starter practices or N  
378 rates in either year, although NIE was greater in 2016 compared to 2017. Ciampitti and Prasad  
379 (2016) found similar mean NIE (42 kg kg<sup>-1</sup>) in high yielding sorghum (>8000 kg ha<sup>-1</sup>).  
380 Moreover, considerable variability was observed in NIE (8-83 kg kg<sup>-1</sup>), similar to the current  
381 study.

382 There was no difference in ANE in regards to starter or increasing rates over the two  
383 years. The mean ANE values ranged from 2.74 – 7.78 in 2016 and 2.37-16.21 in 2017 across  
384 starters and N rates. A two year N rate study on grain sorghum in Nebraska found a decrease in  
385 ANE with increasing N rates from 50-150 kg N ha<sup>-1</sup> applied (Abunyewa et al., 2017).

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**Table 3 Nitrogen Use Efficiency with Contrasting Application Rates.** Average NUE values of starter practices and increasing N rates for grain and biomass in 2016 and 2017. L.S.D. was used to compare means.

	<b>ANE</b>	<b>NRE</b>	<b>NIE</b>	<b>PFP</b>
	kg kg <sup>-1</sup>	%	kg kg <sup>-1</sup>	kg kg <sup>-1</sup>
<b>2016</b>				
<b>Starter</b>				
None	3.72	18.3	32.6	30.49
In-Furrow	4.29	21.4	39.2	32.10
2x2	5.35	40.4	43.9	36.56
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	0.0202
<b>N-Rate</b>				
112	3.69	37.9	46.3	49.34
168	7.78	29.8	27.9	37.25
224	2.74	22.4	27.0	23.91
280	3.51	16.7	53.2	21.69
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	<0.0001
<b>2017</b>				
<b>Starter</b>				
None	16.21	23.16	13.18	46.51
In-Furrow	2.73	35.84	16.17	46.14
2x2	2.37	29.99	11.42	45.69
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	n.s.
<b>N-Rate</b>				
112	10.18	46.35	18.21	71.81
168	7.11	26.37	11.50	47.16
224	4.60	26.79	12.97	35.42
280	3.98	18.14	10.81	28.69
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	<0.0001
n.s. = not significant (>0.05),				

390 **Conclusions**

391           There has been minimal research on how starter fertilizer practices affect grain sorghum  
 392 yield, optimum N rates and NUE in Texas. There are many advantages to split applying N in  
 393 grain sorghum; to increase NUE, reduce environmental concerns, and increase yield (Buah et al.,  
 394 2012; Mahama et al., 2014; Mahama et al., 2016; Tilman et al., 2002.). In the current study, the

395 agronomic optimum N rate for grain sorghum was 168 kg N ha<sup>-1</sup> in 2016 and less than 112 kg N  
396 ha<sup>-1</sup> in 2017. While 2x2 application of starter fertilizer did increase grain yield in one year of the  
397 two-year study, yield enhancement was inconsistent. Furthermore, starter fertilizer practices did  
398 not affect optimum N rates and did not consistently improve NUE in grain sorghum.

399         While the lack of yield response and inability to improve NUE with starter fertilizers in  
400 the current study may bring into question the value of these practices, other factors may have  
401 contributed to the lack of response. The soil tests at the beginning of both years showed P was  
402 sufficient. This could have impacted the N+P starters negatively because the sorghum crop  
403 would not need to assimilate any more P causing economic and environmental loss. A N only  
404 starter would have been more appropriate. High grain yield and high levels of PNU in plots with  
405 no N applied suggests significant residual soil N was present, reducing uptake and use efficiency  
406 of N fertilizer. Mineralization of corn residues from previous crops at the study site likely  
407 contributed to addition plant available N in soil, reducing uptake of fertilizer N. The combination  
408 of these factors likely limited the ability to evaluate the full impact of starter fertilizers on grain  
409 sorghum yield and NUE.



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## CHAPTER III

### STARTER FERTILIZERS AND NITROGEN FERTILIZER TIMING IN GRAIN SORGHUM

#### **Introduction**

Appropriate timing of N fertilizer applications can increase grain yield, NUE, and decrease environmental N loss (Bermudez and Mallarino, 2002; Cassman et al., 2002; Randall et al., 1985; Shrestha et al., 2010; Zotarelli et al., 2015). Split application of N fertilizer can increase NUE compared to single applications (Bermudez and Mallarino, 2002; Gordon and Whitney, 1995; Niehues et al., 2004; Scharf, 1999; Soroka, 2016; Touchton and Karim, 1986). Starter fertilizer applications involve injecting small amounts of N and P fertilizer with or near the seed at planting. For starter fertilizer containing N, the application essentially results in split application of N fertilizer. Application of some portion of N fertilizer with starter fertilizer could influence optimum timing for side-dress N application to grain sorghum. The potential to expand the optimum window for side-dress N application could ease logistical concerns for producers and potentially improve grain yield and NUE in sorghum.

Texas A&M AgriLife Extension recommends to apply all N fertilizer before panicle initiation and subsequent rapid plant growth to maximize grain yield potential (Stichler et al., 1997). Starter fertilizer N applications could be used to meet early season N demands and later side-dress N applications to correspond with rapid growth phases of grain sorghum. While information for split application of N in grain sorghum is limited, many studies have evaluated the impact of split N applications in corn. Miller (2012) demonstrated similar grain yield when N fertilizer was applied at V7 and V15 (leaf stage) when both received started fertilizer. However, Walsh et al. (2012) found applying N once midseason in corn will not compensate for early

433 season N stress with yields reduced up to 15%. For corn, starter fertilizer appears to provide  
434 sufficient N for early season growth and enable later side-dress applications of N without yield  
435 loss. It is uncertain if this strategy will work in grain sorghum.

436 Grain sorghum yield and NUE are likely affected by timing of N fertilizer application.  
437 Starter fertilizers are commonly used in grain sorghum yet little information on the timing of  
438 split N applications is available and how it affects yield components, biomass, and NUE. The  
439 objective of this study is to measure grain sorghum yield in response to nitrogen fertilizer timing  
440 for sorghum with contrasting starter fertilizer practices (no starter, popup and 2x2). In addition,  
441 NUE will be determined for contrasting starter practices with increasing delay of side-dress N  
442 fertilizer application.

#### 443 **Methods and Materials**

444 A two-year study was conducted at the Texas A&M AgriLife Research farm (30.543297,  
445 -96.435316) in Burleson County, TX to evaluate the impact of starter fertilizer on optimum  
446 timing of N fertilizer. The study site is predominately Weswood silty clay loam and Ships clay  
447 under conventional tillage using raised beds.

448 A split-plot design was used with 4 replications and 15 fertilizer treatments. N application  
449 timing (four or five dates) was the main plot and starter fertilizer (three practices) was the sub-  
450 plot. Planting dates of grain sorghum were April 6, 2016 and March 22, 2017, using Dekalb  
451 hybrid DKS 51-01. 160,550 seeds ha<sup>-1</sup> on 76.2 cm spacing using a four-row John Deere 1705  
452 planter. The treatments of the starter solutions were liquid ammonium polyphosphate (11-37-0)  
453 applied at 1) 56 L ha<sup>-1</sup> applied in furrow 2) 168 L ha<sup>-1</sup> placed 5 cm to the side of the seed trench  
454 and up to a 5cm in depth and 3) no starter fertilizer control. Subplots were four rows wide and  
455 20.1 m long in 2016 and 30.5 m in length in 2017. Side-dress N fertilizer timing treatments were

456 applied using a Scotts Turf Builder Classic Drop Spreader to broadcast granular urea (46-0-0) at  
457 a rate of 168 kg N ha<sup>-1</sup> (less N applied through starter practice) between rows. Side-dress N was  
458 applied at approximately the 3-leaf, 6-leaf, 10-leaf, boot, and bloom growth stages. Application  
459 dates are reported as days after planting.

460 Pesticide management strategies followed Agrilife recommendations. In 2016 a pre-plant  
461 burn down consisted of a.i. glyphosate 48.8% (Roundup WeatherMax 2.8 L ha<sup>-1</sup>), a.i. s-  
462 metachlor 82.4% (Dual II Magnum 1.4 L ha<sup>-1</sup>), and a.i. atrazine 42.6% (AAtrex 4L 2.8 L ha<sup>-1</sup>).  
463 Cultivation practices were used early in the season to control weeds. In growth stage 7, a.i.  
464 chlorantraniliprole 5% (Prevathon 1 L ha<sup>-1</sup>) was used to control head insects (*H. zea*, *S.*  
465 *frugiperda*, *Nola sorghiella*, *Nezera viridula*, *Oebalus pugnax*). In 2017 a pre-plant burndown of  
466 a.i. dimethenamid-P 63.9% (Outlook 1 L ha<sup>-1</sup>) and a.i. glyphosate 48.8% (Roundup WeatherMax  
467 2.8 L ha<sup>-1</sup>) was applied before planting. Cultivation practices occurred during growth stage 3 to  
468 remove weeds growing in between the rows in 2017. An in season application of a.i. atrazine  
469 42.6% (AAtrex 4L3.5 L ha<sup>-1</sup>) and a.i. dicamba 48.2% (Rifle 0.58 L ha<sup>-1</sup>) were also applied at  
470 growth stage 4 to suppress broadleaf weeds. An a.i. cyfluthrin 24.74% insecticide (Tombstone 70  
471 mL ha<sup>-1</sup>) was applied to diminish any midge (*Stenodiplosis sorghicola*) infestation during  
472 growth stage 6. An application of a.i. chlorantraniliprole 5% (Prevathon 1 L ha<sup>-1</sup>) and a.i.  
473 lambda-cyhalothrin 12.7% (Silencer 0.21 L ha<sup>-1</sup>) insecticides were applied during growth stage 7  
474 to remove any head insects (*H. zea*, *S. frugiperda*, *Nola sorghiella*, *Nezera viridula*, *Oebalus*  
475 *pugnax*) present.

476 A John Deere 3300 plot combine with a Harvest Master (HM800) on-board weigh system  
477 measured grain yield, moisture, and test weight from the center two rows of each plot. Grain  
478 yield was adjusted to 14% moisture. In 2016 there was poor establishment due to weather. If

479 skips were present they were measured and removed from plot lengths to correct actual harvested  
480 area. All plants from a 3 m section of the first row of each plot was harvested by hand at the soil  
481 surface. It was separated into grain and biomass to measure dry matter and nitrogen content.  
482 Yield components (panicle weight, grain weight, test weight, moisture, and seed size) were  
483 measured to calculate grain yield. The grain was milled (2 mm) with a Wiley Mill 4 and  
484 submitted for measurement of total nitrogen content. Sorghum aboveground biomass was  
485 weighed, chopped in the field using a forage chopper (Case 700), and subsample weighed and  
486 dried (65C) using a forced air oven for 36 hours or until moisture loss was minimal. The dry  
487 matter yield for the biomass was calculated using the Dry matter and moisture. Dried subsamples  
488 were ground using a Wiley Mill 4 (2mm) and sent to the laboratory for tissue N analysis. The  
489 tissue and grain samples collected at hand harvest were submitted to the Texas A&M Soil,  
490 Water, Forage Laboratory to measure total N. Total N (plant tissue and grain) was determined by  
491 high-temperature combustion (McGeehan and Naylor, 1988).

492         Soil cores were taken using a GSRPS Giddings Machine Company soil probe at the  
493 beginning of the growing seasons to determine soil concentration of nitrate-N and extractable  
494 nutrients. Soil was sampled 60 cm in depth and divided into 15 cm segments. The samples were  
495 sent to the Texas A&M Soil, Water, Forage Testing Laboratory for analysis. Mechlich III was  
496 used to extract phosphorus, potassium, calcium, magnesium, sodium, and sulfur and  
497 concentration measured by inductively coupled plasma (ICP) (Mehlich, 1984). Soil pH was  
498 found using a hydrogen selective electrode in a 1:2, soil: deionized water extract. Soil electrical  
499 conductivity was determined using a conductivity probe with the same soil: deionized water ratio  
500 as the soil pH (Schofield and Taylor, 1955). Nitrate nitrogen was extracted from the soil with 1  
501 N KCL solution(Keeney and Nelson, 1982).

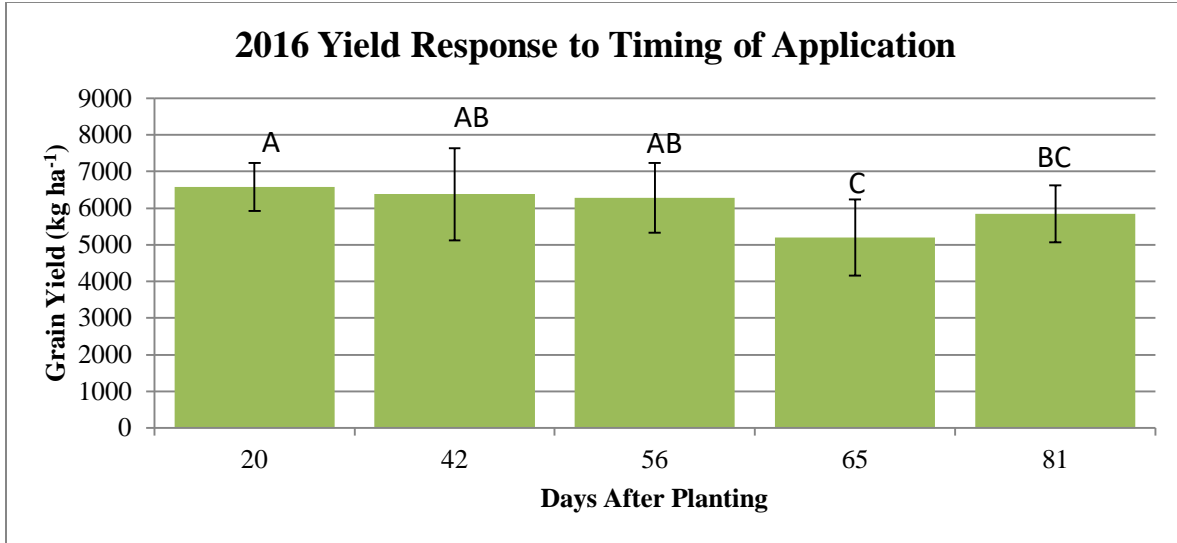
502 Statistical analyses were performed using SAS 9.4 (Cary, NC). The PROC GLM  
503 procedure was used to analyze variable of grain yield, NUE, biomass yield, grain and biomass N  
504 as a split-plot design with fertilizer timing as the main plot and starter fertilizer as the subplot.  
505 When applicable, means were separated using L.S.D. linear and nonlinear regression to identify  
506 optimum side dress timing of N fertilizer.

## 507 **Results and Discussion**

### 508 *Grain and Biomass Yield*

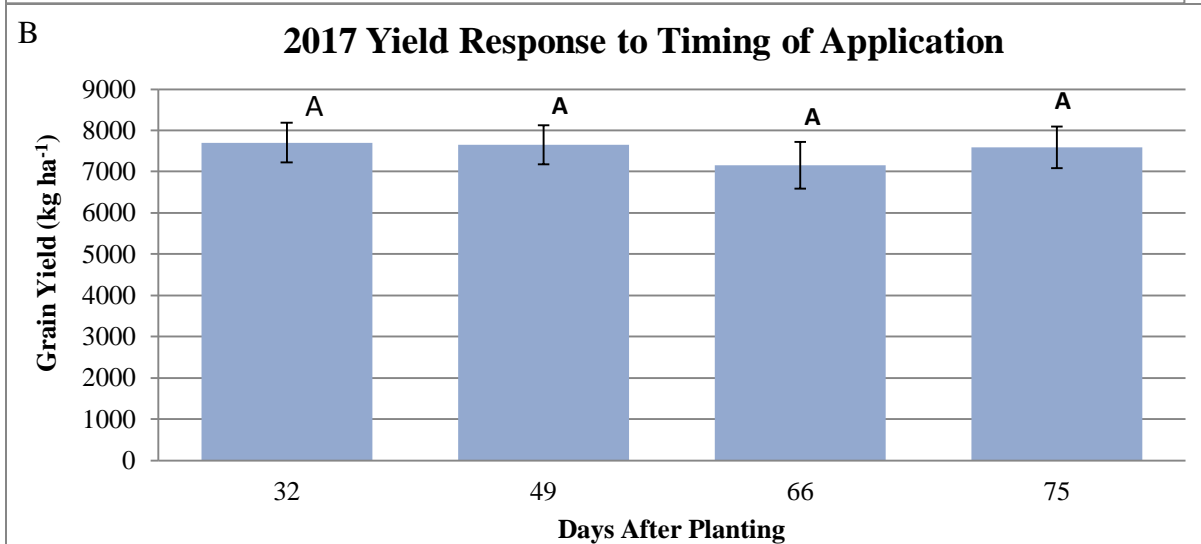
509 Variation in grain yield response to N fertilizer application timing was significant ( $p <$   
510  $0.05$ ) in 2016 and was not significant in 2017. In 2016, grain yield was not reduced when N  
511 was applied within 56 days of planting (Figure 4A). However, no interaction ( $p > 0.05$ )  
512 between starter fertilizer practices and N fertilizer timing was observed. Despite starter  
513 fertilizers having no impact on N timing, yield loss was not observed when N fertilizer was  
514 applied beyond panicle initiation (~30 days after planting). Miller (2012) found similar results  
515 in corn where N fertilizer was applied up to V15 (~55 days after planting) without a decrease  
516 in yield. Grain yield was not affected by N application timing in 2017 (Figure 4B).

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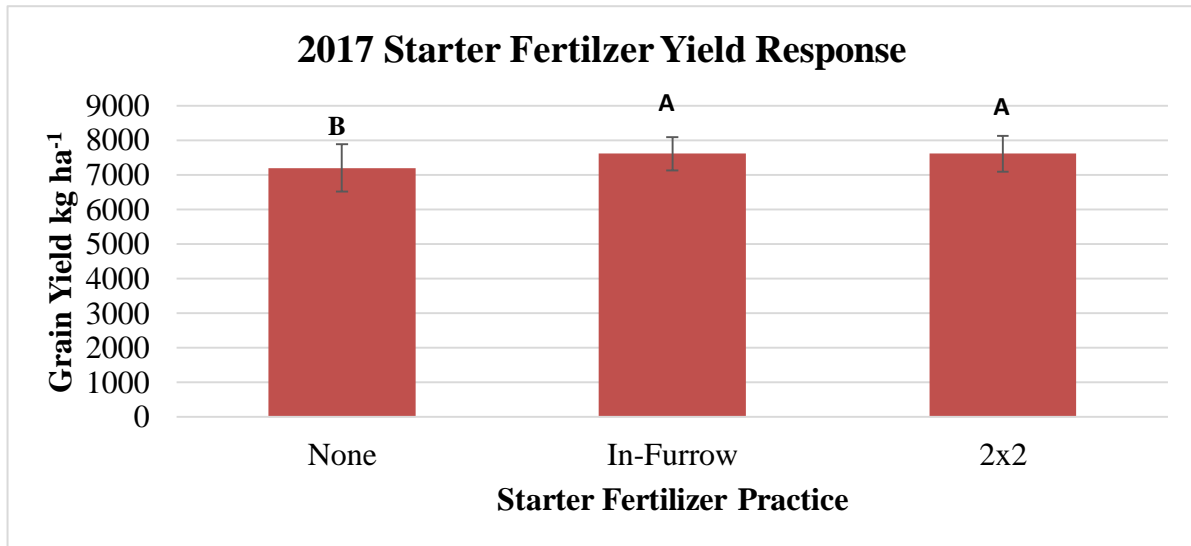
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**Figure 4 A) 2016 Yield Response to Timing of Application B) 2017 Timing Response to Timing of Application.** A and B show the average grain yield response to the application of N side dress timing in 2016 and 2017. The different letters indicate significant difference. Error bars represent one standard deviation.

While starter fertilizer practices did not affect N fertilizer timing, starter fertilizer did



**Figure 5 2017 Starter Fertilizer Yield Response.** Average yield response to starter fertilizer practices. Different letters indicate significant differences in starter fertilizer practices at  $P < 0.05$ . Error bars represent one standard deviation.

525 affect ( $P < 0.007$ ) grain yield in 2017 (figure 5). Starter fertilizer application as 2x2 and in-  
 526 furrow increased yield by 413 to 415 kg ha<sup>-1</sup> compared to grain sorghum without starter  
 527 fertilizer. Walsh et al. (2012) found corn yield increased 15% when N was split applied instead  
 528 of being applied only midseason.

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531 Biomass production between treatments was not significant in 2016 or 2017. The mean in  
 532 2016 was 7,950 kg ha<sup>-1</sup> and in 2017 the mean was 8,171 kg ha<sup>-1</sup>. In contrast, Torbert et al.  
 533 (2001) found with an increase of N applications (0-168 kg N ha<sup>-1</sup>) in two of the three year  
 534 study, when there was adequate rainfall, there was increase in biomass production and N  
 535 uptake under three tillage systems (no-till, chisel no-beds, and chisel with beds). In a sweet

536 sorghum N management trial (Almodares and Darany, 2006) found the best timing of  
537 application to be between the 3-8 leaf stages for highest biomass production compared to  
538 applying N at boot or soft dough stage.

539 The lack of response to the timing of application could have been due to sufficient levels  
540 of N and P in the soil. Residual soil nitrate nitrogen averaged 19 kg ha<sup>-1</sup> in the top 15 cm in  
541 2016. In 2017, there was an average of 8 kg ha<sup>-1</sup> in the top 15 cm and 46 kg ha<sup>-1</sup> in the top 60  
542 cm. This could have caused the sorghum to have adequate levels of N and P when the starter  
543 was applied. Both years of the study followed a high yielding corn crop. Nitrogen could have  
544 been added into the system through mineralization of the corn residue. Aulakh et al. (1991)  
545 found in a laboratory study that corn residues added 27 mg kg<sup>-1</sup> of N to the soil. In a field study  
546 in Canada, Burgess et al. (2002) found 70-80% of N in corn residue was released into the soil  
547 over two years. In the current study there was no affect on the timing of the N application out  
548 to 75 days after planting in 2017, this could be due to the effect of mineralization of organic N  
549 from the corn residue adequately supplying the sorghum. Therefore, the application of 168 kg  
550 N ha<sup>-1</sup> may have not been assimilated into the sorghum.

#### 551 *Plant Nitrogen Concentration and Uptake*

552 Sorghum biomass N concertation was not affect by fertilizer timing in 2016 or 2017 and  
553 starter fertilizer practices affected (p<0.05) sorghum biomass N concentration in 2017 only  
554 (Table 4). Mean biomass N concentration was 0.99% in 2016 and 1.27% in 2017. Starter  
555 fertilizers increased sorghum biomass N concentration 8.2% compared to sorghum without  
556 starter fertilizer in 2017. Similar to sorghum biomass, grain N concentration was not affected by  
557 N fertilizer timing or starter practice in either year.



558 Nitrogen fertilizer application timing and starter practices did not result in significant  
 559 variation in sorghum biomass in 2016 or 2017 and differences in grain yield in 2016 only.  
 560 Therefore, uptake of N by sorghum biomass was not affected by N fertilizer timing of starter  
 561 fertilizer practices in either year. In contrast, as N fertilizer timing was delayed in 2016, N uptake  
 562 by grain decreased. There was a 20% decrease in grain N uptake when N fertilizer was applied at  
 563 65 days after planting compared to 42 days after planting. The impact of N fertilizer timing was  
 564 negligible on grain N uptake in 2017. As a result of variation in biomass production, grain yield  
 565 and N concentration in plant tissues, total plant N uptake was not affected by N timing in 2016 or  
 566

567 **Table 4 Plant Nitrogen Concentration and Nitrogen Uptake with Delayed Nitrogen Application.** Average N  
 568 concentration and N uptake of starter practices and N application timings for grain and biomass in 2016 and 2017.  
 569 L.S.D was used to compare means.

	N Concentration (g kg <sup>-1</sup> )		N Uptake (kg ha <sup>-1</sup> )		
	Biomass	Grain	Biomass	Grain	PNU
<b>Starter</b>	<b>2016</b>				
None	9.9	15.5	80.6	80.3	160.9
In-Furrow	9.6	15.7	73.7	77.2	150.8
2x2	10.1	15.2	77.8	83.1	160.9
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	n.s.	n.s.
<b>N-Timing</b>					
20	9.4	15.0	72.7	84.6	157.3
42	10.3	15.9	79.5	86.6	166.0
56	9.9	15.7	79.7	84.3	164.0
65	9.6	15.5	70.2	68.8	139.0
81	10.3	15.4	84.7	76.8	161.6
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	0.0044	n.s.
<b>Starter</b>	<b>2017</b>				
None	8.5	12.6	86.8	77.0	170.1
In-Furrow	9.2	12.8	110.6	82.8	205.5
2x2	9.3	12.8	113.6	82.6	206.2
<i>Pr &gt; F</i>	0.005	n.s.	n.s.	0.017	0.041
<b>N-Timing</b>					
32	8.6	12.6	110.5	84.8	195.3

49	8.8	12.9	88.5	85.6	174.1
66	9.0	13.0	140.1	80.7	220.8
75	9.7	12.4	103.8	81.8	185.6
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	n.s.	n.s.
n.s. = not significant (>0.05), PNU (total plant N uptake)					

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572 2017. Starter fertilizers did increase PNU by 20% in 2017 compared to sorghum without starter  
573 fertilizer.

574 While N fertilizer timing had no impact in sorghum N concentration and N uptake, starter  
575 fertilizers practice did influence N concentration and uptake in 2017. Starter fertilizer essential  
576 result in split application of N and did improve PNU compared to sorghum without starter  
577 fertilizer. Similar results were found for corn where PNU was increased when N was split  
578 applied compared to single applications (Niaz et al., 2014). The lack of sorghum response to N  
579 timing may have been influenced by residual soil N and mineralization of previous crop residues.  
580 Delayed N application would be expected to impact grain and biomass yield when N is limiting.

581 *Nitrogen Use Efficiency*

582 Nitrogen use efficiency in response to N fertilizer timing and starter fertilizers were  
583 evaluated during 2017. Timing of N application had no effect ( $P > 0.05$ ) on NUE (Table 5).  
584 However, starter fertilizer practices did increase ( $p < 0.05$ ) NRE compared to sorghum without  
585 starter fertilizer. There was greater than 2-fold increase in NRE for sorghum with in-furrow or  
586 2x2 starter applications compared to sorghum without starter. While NRE was improved with  
587 starter fertilizer applications, and likely associated with split N applications, NRE was lower than  
588 reports for other crops with split applied N (Kovács et al., 2015a)

589 Duan et al. (2014) found greater ANE values when N and P was applied compared to N  
 590 alone in a long-term (1995-2001) fertilizer management study for a wheat and corn rotation.  
 591 When P was applied with N, ANE increased for wheat from 1 to 19 kg kg<sup>-1</sup> (kg grain per kg  
 592 nitrogen) and from 2 to 19 kg kg<sup>-1</sup> for corn. In our study, we did not observe a difference in ANE  
 593 due to starter applications, which provide N and P. However, our field was not limiting in soil  
 594 test P and likely limited the impact of P applications. As previously stated, starter applications do  
 595 provide an opportunity to split apply N which did result in greater NRE during 2017.  
 596

597 **Table 5 Nitrogen Use Efficiency with Nitrogen Application Timing.** Average NUE values of starter practices and  
 598 N application timings for grain and biomass in 2017. L.S.D. was used to compare means.

	ANE	NRE	NIE	PFP
	kg kg <sup>-1</sup>	%	kg kg <sup>-1</sup>	kg kg <sup>-1</sup>
<b>Starter</b>				
<b>2017</b>				
None	3.10	18.74	7.07	43.44
In-Furrow	5.23	39.79	21.07	45.57
2x2	5.01	40.21	8.56	45.35
<i>Pr &gt; F</i>	n.s.	0.0407	n.s.	n.s.
<b>N-Rate</b>				
32	5.52	33.7	16.88	45.86
49	5.20	21.10	30.28	45.53
66	2.25	48.89	-2.25	42.58
75	4.84	27.96	4.05	45.17
<i>Pr &gt; F</i>	n.s.	n.s.	n.s.	n.s.
n.s. = not significant (>0.05), PNU (total plant N uptake)				
	Agronomic Nitrogen Efficiency (ANE) $= \frac{Y_n - Y_o}{\text{applied N rate}}$	Nitrogen Recovery Efficiency (NRE) $= \frac{PNU_n - PNU_o}{\text{applied N rate}} \times 100$	Nitrogen Internal Efficiency (NIE) $= \frac{Y_n - Y_o}{PNU_n - PNU_o}$	Partial Factor Productivity (PFP) $= \frac{\text{yield}}{\text{applied N rate}}$

599 **Conclusions**

600 Application of a starter fertilizer with N could affect optimum timing for side-dress N  
601 application to grain sorghum. Possibly expanding the window of the side-dress N application  
602 could minimize concerns for producers who cannot get into the field early in the season. The  
603 timing of side dress N application is crucial in grain sorghum to have high yields, improved  
604 NUE, and is important for environmental factors (Bermudez and Mallarino, 2002; Cassman et  
605 al., 2002; Randall et al., 1985; Shrestha et al., 2010; Zotarelli et al., 2015). In the current study,  
606 starter fertilizers did not have an effect on the timing of side dress N in either year of the study.  
607 In 2016, N fertilizer could be applied up to 56 days after planting before the grain yield was  
608 impacted negatively. In 2017, the timing of the side dress did not affect grain yield, which could  
609 have been due to other factors.

610 For sorghum without N fertilizer 2017, high PNU and substantial grain yield was  
611 observed. This indicates there were high levels of plant available N in soil. Soil tests taken before  
612 planting indicated sufficient levels of P and significant levels of residual N, which exceeded the  
613 amount of N and P in the starter fertilizers. This likely contributed to the lack of response to  
614 starter fertilizer and the insignificant impact on the side-dress N timing. Moreover, uptake of N  
615 plots without N fertilizer exceeded the N found in the soil test. This indicates additional sources  
616 of N were found within the system. The study site used in both years followed high yielding  
617 corn, ranging from 11,300 kg ha<sup>-1</sup> to 12,550 kg ha<sup>-1</sup>. Mineralization of corn residue supplied  
618 additional plant available N throughout the growing season. With significant levels of residual  
619 soil N and mineralizable N, the ability to detect differences among starter fertilizers and their  
620 impact on timing of side-dress N in the grain sorghum was diminished.

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## CHAPTER IV

### EVALUATION OF CONTRASTING SENSING TECHNOLOGY TO DETECT NITROGEN STRESS IN GRAIN SORGHUM

#### **Introduction**

Efficient monitoring of nitrogen status in grain sorghum can lead to improved N fertilizer management. (Graeff and Claupein, 2003; Singh et al., 2017; Wang et al., 2017; Zhao et al., 2005). A variety of sensors are commercially available that enable plant or canopy level measurements of field crops at increasing scales (Zhou et al., 2016). Nitrate selective electrodes have been used successfully to monitor nitrogen status of individual plants from petiole sap extruded from a variety of crops (Hartz et al., 1993; Hochmuth, 1994; Krusekopf et al., 2002). Similarly, hand held sensors have been used to estimate leaf chlorophyll content from reflectance readings at points on individual leaves (Bullock and Anderson, 1998). Additional developments in technology have enabled canopy level reflectance measurements of many crops using ground based proximal sensing. Recently, advancements in remote sensing technology has enabled field level measurement of crop canopy reflectance using unmanned aerial vehicles (UAV) or satellite systems. While technology has advanced and is readily available to producers, limited information is available for use of this technology to monitor and manage N in grain sorghum.

Many studies have successfully monitored N status in a variety of crops using leaf or canopy level reflectance measurements (Graeff and Claupein, 2003; Zhao et al., 2005). Many spectral bands and algorithms derived from a variety of spectral bands have been proposed to accurately monitor various canopy characteristics. Graeff and Claupein (2003) showed that N deficiency in corn plants can be correlated with leaf reflectance values in the near- infrared band

644 (750- 1350nm). Zhao et al. (2005) reported correlation between leaf N status and chlorophyll  
645 content to leaf reflectance near 550 nm and 710 nm for N deficient sorghum. Singh et al. (2017)  
646 found increasing leaf reflectance values in the green and red bands with increasing N fertilizer  
647 rates in sweet sorghum.

648         Of the numerous spectral bands and algorithms that have been developed to measure N  
649 status in crops, normalized difference vegetative index (NDVI) is one of the most frequently  
650 used vegetative indices to monitor crop canopies (Foster et al., 2017; Wang et al., 2017).  
651 Canopy level NDVI has been correlated with crop biomass, grain yield and N status (Foster et  
652 al., 2017; Lai-gang et al., 2011; Ma et al., 1996; Rambo et al., 2010b; Wang et al., 2016). Lai-  
653 gang et al. (2011) was able to correlate leaf N content from wheat with NDVI derived from a  
654 SPOT-5 satellite image. Ma et al. (1996) found NDVI (obtained from CropScan) to detect  
655 increasing N rates (0, 100, and 200 kg N ha<sup>-1</sup>) throughout the growing season in a corn N  
656 management study. Wang et al. (2016) calculated NDVI from Landsat 5 TM images over a 10-  
657 year period and found a strong correlation between NDVI and corn grain yield at pre-silking.  
658 Indicating the crop stress is related to grain yield, whether it is due to N deficiency or  
659 environmental factors. Moreover, field level NDVI readily available to producers through many  
660 commercial providers of remote sensing data. NDVI is calculated as:

$$\text{NDVI} = \frac{(\text{NIR} - \text{Red})}{(\text{NIR} + \text{Red})}$$

661 NDVI values range between -1 and 1 with green vegetation being positive values. Wang et al.  
662 (2017) measured seasonal variation of NDVI for wheat canopies and correlated N leaf status  
663 with NDVI at three growth stages (jointing, boot, and anthesis). In addition, NDVI was found to  
664 be highly correlated N status of biomass sorghum (Foster et al., 2017). This suggests that

665 monitoring N status in grain sorghum using proximal or remote sensing platforms may be  
666 possible.

667         Handheld multispectral radiometers can measure the canopy reflectance. CropScan  
668 (MSR85, CropScan Inc., Rochester, MN) is a hand held or vehicle mounted proximal sensor that  
669 is configured with eight wavelengths (460-810nm). CropScan has been used extensively to  
670 measure canopy reflectance in response to crop N status (Ma et al., 1996; Osborne et al., 2002;  
671 Rambo et al., 2010a; Shanahan et al., 2001). Rambo et al. (2010a) found CropScan could detect  
672 N differences in corn among increasing rates (0-240 kg N ha<sup>-1</sup>). This was reported only during  
673 mid-season (v10), not early (v6) or late season (R1). Ma et al. (1996) used CropScan to  
674 distinguish between N treatments (0,100, and 200 kg N ha<sup>-1</sup>) in corn. They found treatment  
675 effects over three collection dates (preanthesis, anthesis, postanthesis) which could help predict  
676 N deficiency throughout the growing season or predict potential grain yield. Ma et al. (1996)  
677 also found a strong correlation between NDVI, obtained using CropScan, and field greenness (a  
678 product of chlorophyll meter readings [SPAD] and leaf area). Detection of N stress in grain  
679 sorghum near growing point differentiation, and certainly before flowering, will be essential to  
680 enable corrective actions.

681         In addition to canopy reflectance, chlorophyll meters have been used to detect N  
682 deficiency in plants (Wu et al., 2007). The hand held SPAD 502 chlorophyll meter is a  
683 spectrophotometer that measures absorption of red and infrared wavelength at points on  
684 individual leaves, which is known to correspond with chlorophyll levels in the leaf (Bullock and  
685 Anderson, 1998; Reyes et al., 2017; Uddling et al., 2007; Yu et al., 2010; Yu et al., 2012). In  
686 addition to chlorophyll estimation, chlorophyll meters are capable of differentiating between N  
687 sufficient and deficient corn (Rambo et al., 2010a). While manual measurements from individual

688 plants may not be the most efficient way to assess field variation of crop N status, it could be an  
689 essential, nondestructive method for confirming remotely sensed data.

690 In contrast to nondestructive methods, plant nitrogen status can be assessed through  
691 measurement of petiole sap nitrate using a compact nitrate ion meter (NIM) (Wu et al., 2007).  
692 The NIM is a low cost, handheld nitrate ion specific electrode with a digital display. Plant  
693 petioles or midribs are removed and sap extruded using a device similar to a garlic press for  
694 measurement in the NIM electrode. NIM has been used in many vegetable and fruit crops to  
695 record the petiole sap nitrate concentration throughout the growing season (Anderson et al.,  
696 2016; Deitch, 2016; Hochmuth, 1994; Zhang et al., 1996). Although some tissue is removed for  
697 sap nitrate measurement, the test provides a quick low cost alternative to laboratory analysis.  
698 Moreover, NIM could be used to compliment proximal and remotely sensed field crops.

699 Many sensors are available for monitoring N status throughout the growing season for  
700 grain sorghum. Yet, minimal research has been done in Texas to monitor the canopy reflectance,  
701 and N status in grain sorghum. The objective of this study is to evaluate sorghum leaf and  
702 canopy level reflectance under contrasting starter fertilizer and N management practices. Relate  
703 leaf and canopy reflectance to midrib nitrate-N concentration, whole plant tissue N  
704 concentration, crop biomass and grain yield.

## 705 **Materials and Methods**

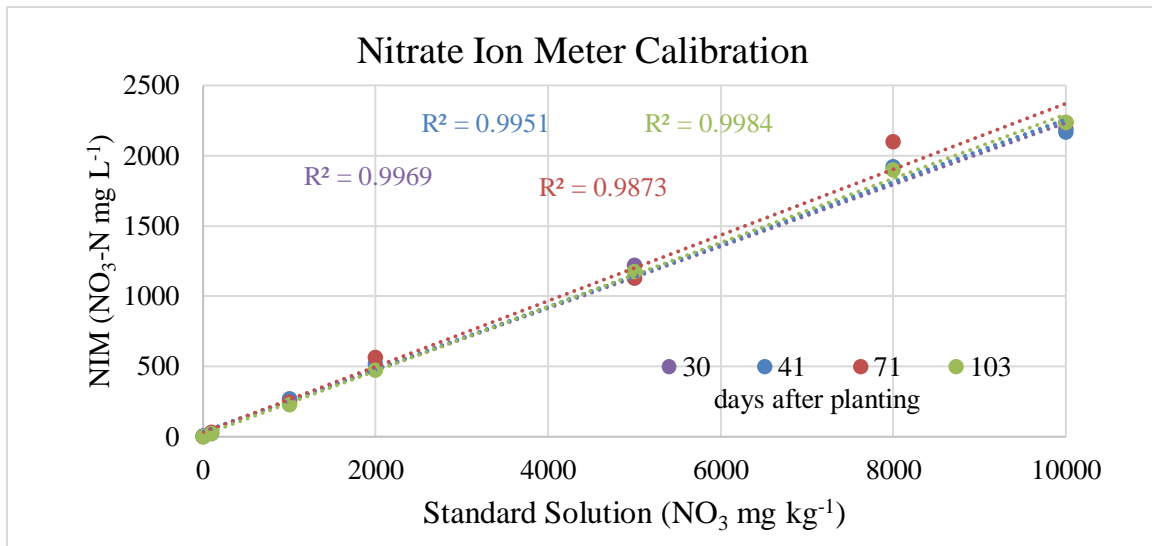
706 A two-year field study was led in Burleson County, TX at the Texas A&M AgriLife  
707 Research farm (30.543297, -96.435316) to evaluate crop sensors for grain sorghum under  
708 contrasting levels of N management. The plot design is described in detail in chapter two.  
709 Briefly, the study was arranged in a split plot design with four replications of fifteen fertilizer  
710 treatments for grain sorghum production during 2016 and 2017 growing season. Nitrogen rate (0,



711 112, 168, 224, 280 kg N ha<sup>-1</sup>) was the main plot and the starter fertilizer practice (none, in  
712 furrow, 2x2) as the sub-plot. The treatments of the starter solutions were liquid ammonium  
713 polyphosphate (11-37-0) applied at 56 L ha<sup>-1</sup> (in furrow), 168 L ha<sup>-1</sup> (2x2) and no starter. Grain  
714 sorghum was planted on April 6, 2016 and March 22, 2017, both years using sorghum hybrid  
715 Dekalb DKS 51-01. The seeding rate was 160,550 seeds ha<sup>-1</sup> using a four-row John Deere 1705  
716 planter on 76.2 cm spacing. Plots were 3 m wide and 20.1 (2016) and 30.5 (2017) m in length.  
717 Contrasting sensors were used to monitor canopy reflectance and N status of grain sorghum  
718 throughout the growing season over a two-year period. Sensors evaluated included CropScan,  
719 SPAD, and NIM.

720 CropScan (Rochester, MN) was used four times in 2016 (49, 70, 93, and 113 days after  
721 planting) and five times in 2017 (32, 41, 83, 106, and 118 days after planting) to measure canopy  
722 reflectance at 1.2 m above the canopy. All of the measurements were made between 10 am and 2  
723 pm from the center two rows. In 2016, measurements were taken in point mode with reflectance  
724 values averaged for a single point in each plot. In 2017, measurements were taken in continuous  
725 mode, scanning the full length of each plot and generating the average reflectance for each plot.  
726 Continuous mode was used to capture variation within plots..

727 The Soil-Plant Analyses Development (SPAD) chlorophyll meter (Konica Minolta Inc,  
728 Osaka, Japan) was used to measure reflectance from points on 10 random leaves for each plot.  
729 There were four collection dates (30, 41, 71, 103 days after planting) with readings occurring  
730 between 10 am and 2 pm. For randomly sampled leaves in each plot, SPAD readings were taken  
731 at the mid-point of the most recently matured leaf. A standard reference reflectance disk was  
732 measured every 10 plots. Mean SPAD values for each plot are recorded.



733 From the same ten leaves used for SPAD measurements, leaf samples were collected for  
 734 measurement of sap nitrate-N using a NIM (Horiba LAQUAtwin B-741, Kyoto, Japan). Leaf  
 735 samples were kept on ice from the time of collection until NIM measurements. All NIM  
 736 measurements were under laboratory conditions. The midrib was separated from each leaf and  
 737 sap was expelled using a garlic press into a sample cup. Approximately 1 ml of sap was applied  
 738 to the NIM electrode for measurement of NO<sub>3</sub>-N concentration. Standard solutions (100 to  
 739 10,000 mg NO<sub>3</sub> kg<sup>-1</sup>) were prepared using KNO<sub>3</sub> for calibration of the NIM (Figure 6). Standard  
 740 checks of 2,000 and 5,000 mg NO<sub>3</sub> kg<sup>-1</sup> were included every 10 samples for sap nitrate  
 741 measurement.

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**Figure 6 Nitrate Ion Meter Calibration.** Correlation between the NIM reading and standard solution at each collection date. P < 0.001 for all dates.

747 A John Deer 3300 plot combine equipped with a Harvest Master (HM800) weigh system  
748 was used to measure grain yield, moisture, and test weight and were taken from the center two  
749 rows of each plot. Grain yield was adjusted to 14% moisture. In 2016 there were skips, due poor  
750 establishment. The skips were measured and deducted from plot lengths to correct actual  
751 harvested area. Plants were harvested by hand (cut at soil level) in a 3 m section of the first row  
752 of each plot. The plants were separated into grain and aboveground biomass to measure dry  
753 matter and N content. From the hand harvest panicle weight, grain weight, test weight, moisture,  
754 and seed size was measured to calculate grain yield. The grain was milled (2 mm) using a Wiley  
755 Mill 4 and submitted, to the Texas A&M Soil, Water, Forage Laboratory, for measurement of  
756 total nitrogen content. The aboveground biomass was weighed, chopped in the field using a  
757 forage chopper (Case 700), and subsample weighed and dried (65C) using a forced air oven for  
758 36 hrs or until moisture loss was minimal. Dry matter and moisture content from the biomass  
759 was used to calculate dry matter yield for aboveground biomass. Dried biomass subsamples were  
760 milled using a Wiley Mill 4 (2mm) and sent to the laboratory for N analysis. The tissue and grain  
761 samples collected at hand harvest were submitted to the Texas A&M Soil, Water, Forage  
762 Laboratory to measure total nitrogen in biomass and grain samples. Total N (plant tissue and  
763 grain) was determined by high-temperature combustion (McGeehan and Naylor, 1988).

764 Soil cores were collected before the growing season of both years using a GSRPS  
765 Giddings Machine Company soil probe. Soil was sampled in 15 cm segments up to 60 cm in  
766 depth. The samples were sent to the Texas A&M Soil, Water, Forage Testing Laboratory for  
767 analysis. Mechlich III was used to extract phosphorus, potassium, calcium, magnesium, sodium,  
768 and sulfur and concentration measured by inductively coupled plasma (ICP) (Mehlich, 1984). A  
769 hydrogen selective electrode was used to determine soil pH in a 1:2, soil: deionized water

770 extract. A conductivity probe was used to find soil electrical conductivity with the same soil:  
771 deionized water ratio as the soil pH (Schofield and Taylor, 1955). Nitrate N was extracted from  
772 the soil with 1 N KCL solution (Keeney and Nelson, 1982).

773 Statistical analyses were performed using SAS 9.4. The PROC GLM procedure was used  
774 to analyze variable of grain yield, NUE, biomass yield, grain and biomass N, CropScan NDVI,  
775 SPAD, and leaf nitrate content as a split-plot design with fertilizer rate as the main plot and  
776 starter fertilizer as the subplot. When appropriate, means were separated using L.S.D. The PROC  
777 CORR procedure was used to form a correlation matrix between variables grain and biomass  
778 yield, NUE, grain and biomass N, CropScan NDVI, SPAD, and leaf nitrate content. This was  
779 separated by date when necessary.

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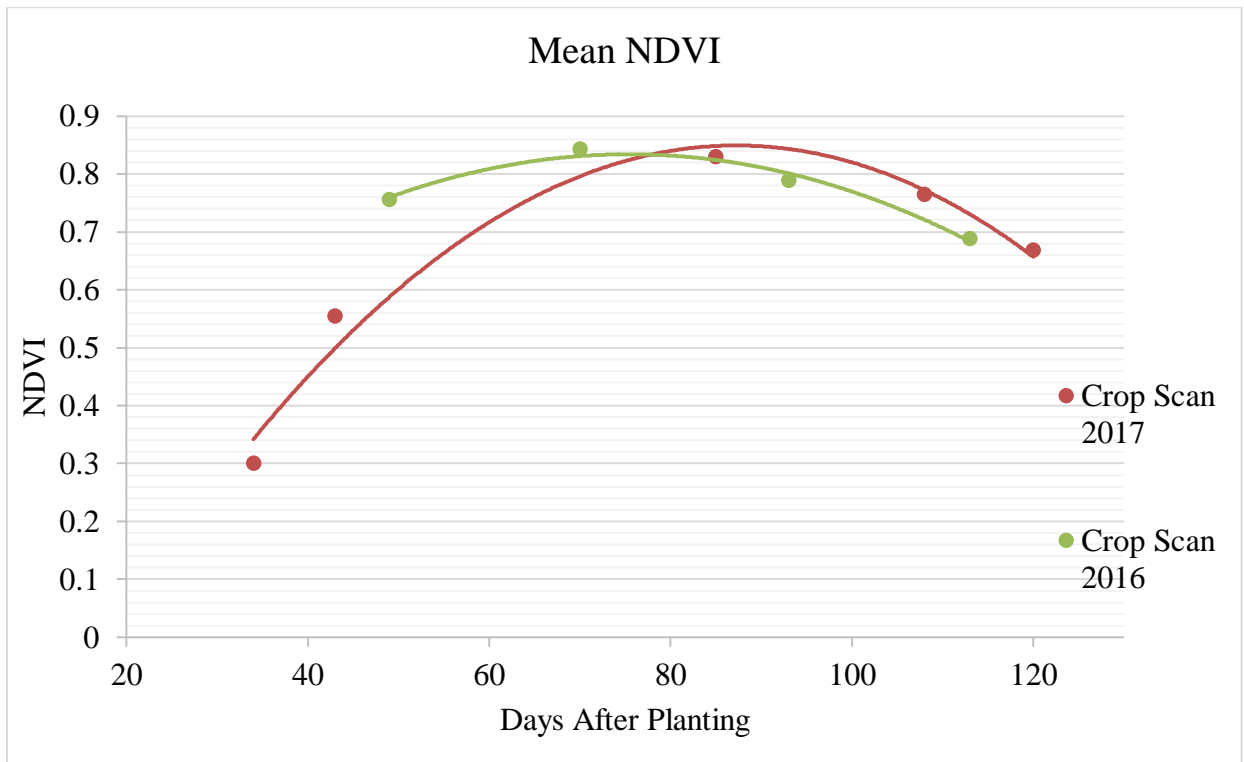
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## 783 **Results and Discussion**

### 784 *Canopy Reflectance - Normalized Difference Vegetative Index*

785 Average NDVI was determined for grain sorghum in response to fertilizer treatments  
786 using CropScan in 2016 and 2017 (Figure 7). Mean NDVI values were fit to a nonlinear  
787 Gaussian model to estimate seasonal variation of NDVI. In 2016, NDVI measured using  
788 CropScan peaked at 70 days after planting and decreased until harvest. 2017 CropScan NDVI  
789 peaked at 87 days after planting with mean NDVI declining through harvest. Seasonal variation  
790 in NDVI for grain sorghum, measured using CropScan, followed similar patterns reported for  
791 other crops (Kim et al., 2015; Moges et al., 2007). Peak NDVI for grain sorghum in 2017 was 87  
792 days after planting, which was 11 days after flowering occurred. Maximum seasonal NDVI is

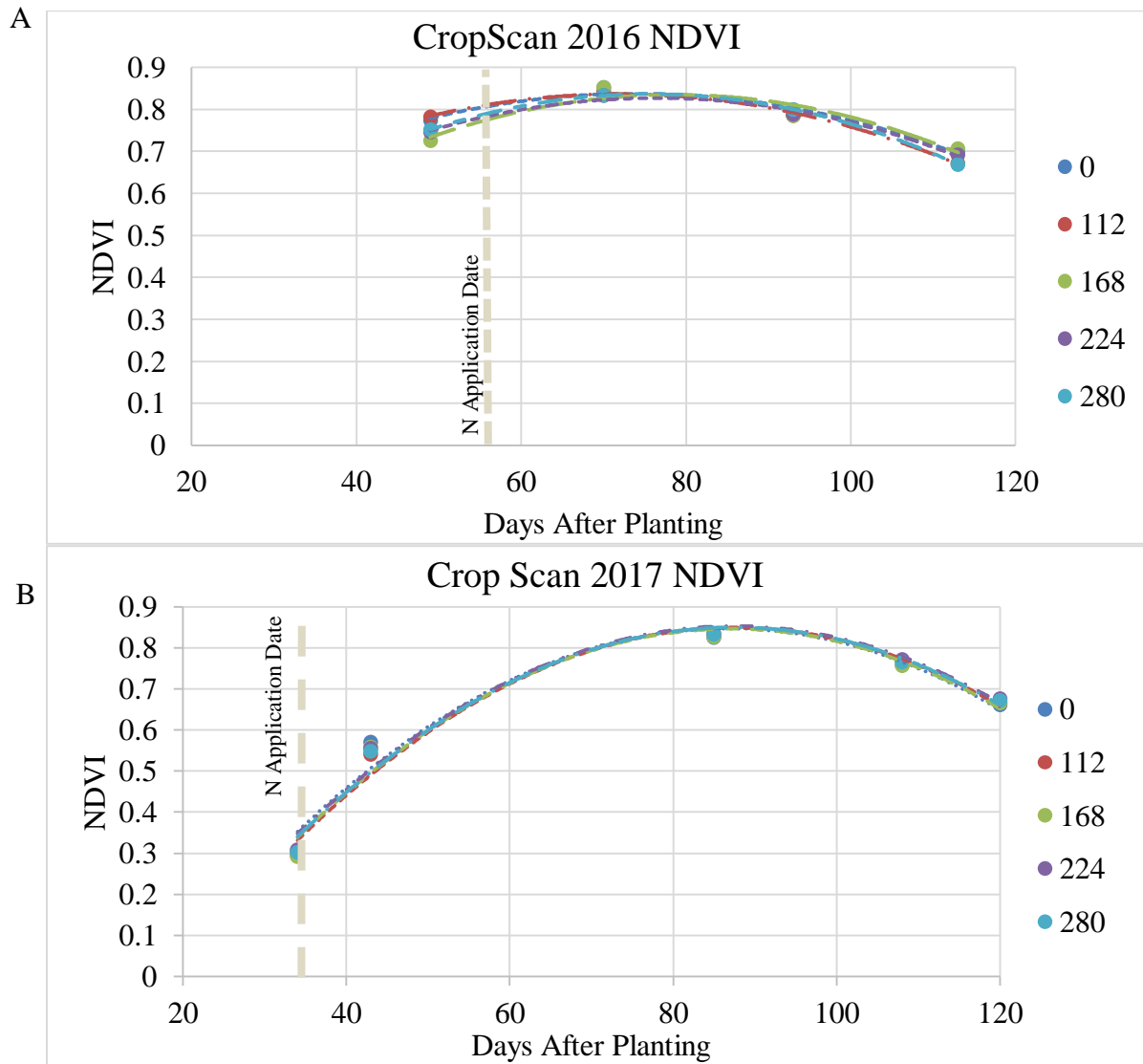
793 associated with maximum plant biomass and leaf area, which occurs near flowering for corn  
794 (Hatfield, 2014).



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796 **Figure 7 Mean Normalized Difference Index (NDVI).** Average NDVI for the 2016 CropScan and 2017 CropScan  
797 over the growing season.

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800 Although seasonal variation of NDVI measured using CropScan followed expected  
801 patterns, there was not a significant difference ( $p > 0.01$ ) between N rates at any date during the  
802 growing season in 2016 or 2017 (Figures 8A & 8B). The lack of N stress in the field likely  
803 limited the ability to detect differences between increasing N fertilizer rates using NDVI. Moges  
804 et al. (2007) found similar results when evaluating NDVI and grain sorghum response to N  
805 fertilizer. Residual soil N and mineralization of N in crop residues limit sorghum response to N  
806 fertilizer therefore precluding comparison of NDVI for increasing N fertilizer rates. However,  
807 Ma et al. (2005) demonstrated significant variation of NDVI for corn under N limiting situations.

808 Grain sorghum NDVI in response to N fertilizer inputs should be evaluated under N limiting  
809 conditions to assess the potential for N fertilizer management.



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812 **Figure 8 A) CropScan 2016 Normalized Difference Index (NDVI) B) CropScan 2017 NDVI.** Average NDVI  
813 for the 2016 CropScan and 2017 CropScan over the growing season with increasing N rates. Application date was  
814 56 days after planting in 2016 and 34 days after planting. Legend units are kg N ha<sup>-1</sup>.

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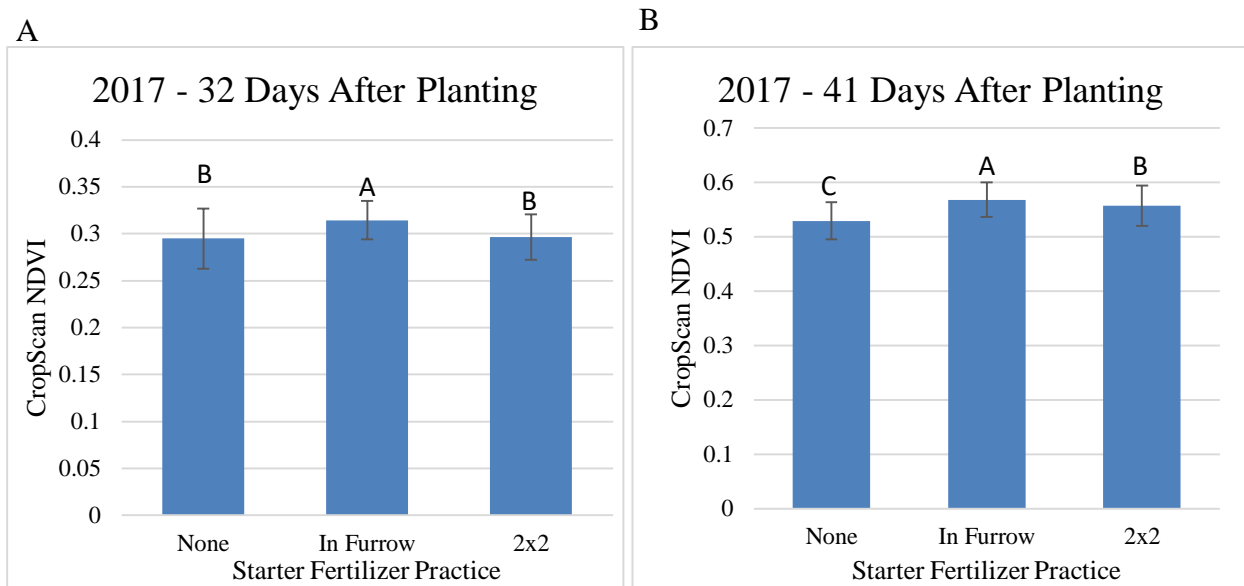
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817 Despite the lack of response to N fertilizer rates, NDVI measured using CropScan did

818 detect differences ( $p < 0.05$ ) for contrasting starter fertilizer practices during 2017. At 32 days

819 after planting, grain sorghum with in-furrow starter fertilizer had greater ( $p < 0.05$ ) NDVI

820 compared to the 2x2 and no starter applied practices (Figure 9A). At 41 days after planting, 2x2  
821 and in-furrow starter fertilizer applications resulted in greater NDVI ( $p < 0.001$ ) than sorghum  
822 without starter fertilizer (Figure 9B). Placement of starter fertilizer, 2x2 or in-furrow, may have  
823 contributed to differences between starter fertilizers at 32 DAP and which was reduced by 41  
824 DAP. Variation of NDVI was negligible among starter fertilizer practices by 83 days after  
825 planting.  
826



**Figure 9 A) 2017 CropScan NDVI – 32 Days After Planting B) 2017 CropScan NDVI – 41 Days After Planting.** Average NDVI of starter fertilizer treatments. Letters above bars indicate significant difference of starter practices at  $p < 0.05$ . Errors bars represent one standard deviation.

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830 Previous studies have shown correlation of sorghum grain yield and grain N content with

831 NDVI as well other vegetation indices (Moges et al., 2007). Increasing N rates did increase grain

832 yield and PNU in 2016 (table 5). Yet, NDVI measured with CropScan did not correlate with

833 grain yield or PNU in the current study. Grain yield and PNU were greater for sorghum with no  
 834 N applied in the current study compared to previous studies. Reduced variability in grain yield  
 835 may have been due to significant amounts of residual N in the soil and mineralization of previous  
 836 crop residues. This likely reduces the magnitude of NDVI differences measured across N rates  
 837 and prohibits correlation with grain yield.

838

839 **Table 6 Yield and Plant Nitrogen Uptake for 2016 and 2017.** Average yield and PNU for contrasting N  
 840 management practices. L.S.D. was used to compare means.

	Yield (kg ha <sup>-1</sup> )		PNU ( kg ha <sup>-1</sup> )	
	2016	2017	2016	2017
<b>Starter</b>				
None	5201	7863	156.4	169.5
In-Furrow	5515	7964	137.5	175.1
2x2	6305	7931	151.5	182.4
<i>Pr &gt; F</i>	0.0022	n.s.	n.s.	n.s.
<b>N-Rate</b>				
0	5156	7672	117.5	140.5
112	5526	8043	146.8	175.5
168	6258	7925	157.8	179.0
224	5356	7934	161.1	197.3
280	6072	8032	159.1	190.4
<i>Pr &gt; F</i>	<0.01	n.s.	<0.01	n.s.
n.s. = not significant (>0.1), PNU (total plant N uptake)				

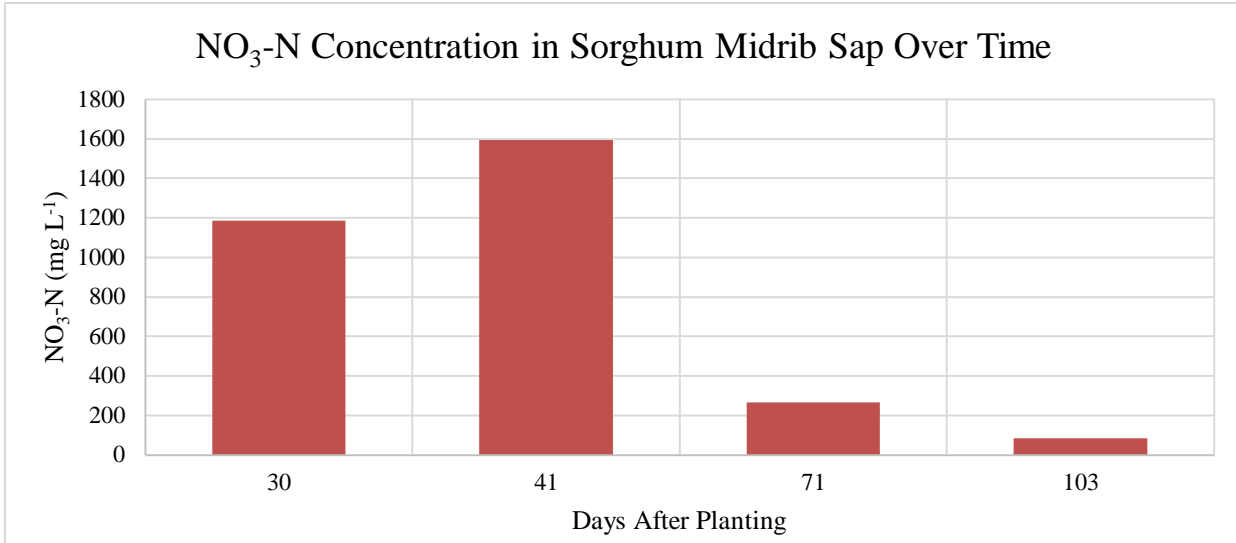
841 *Handheld Sensors*

842 Nitrate Nitrogen Concentration in Sorghum Midrib Sap

843 Sorghum midrib sap was obtained from the most recently mature leaf at four dates in  
 844 2017 for measurement of NO<sub>3</sub> concentration (Figure 10). Seasonal variation of NO<sub>3</sub>-N  
 845 concentration in sorghum midrib sap followed a sigmoidal pattern with increasing concentration  
 846 leading up to flowering and declining concentrations through grain fill. Sorghum sap NO<sub>3</sub>-N



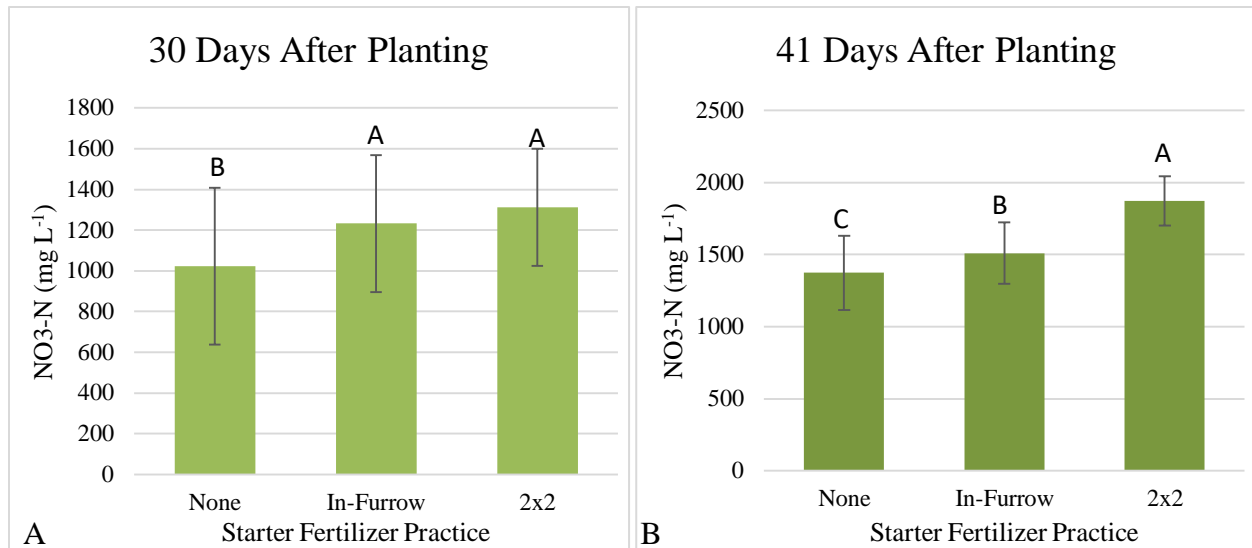
847 concentration were greatest at 41 days after planting with a mean of 1593 mg L<sup>-1</sup> and was lowest  
848 at grain fill with a mean concentration of 83 mg L<sup>-1</sup>.



**Figure 10 NO<sub>3</sub>-N Concentration in Sorghum Midrib Overtime.** Average NO<sub>3</sub>-N from the NIM over the growing season.

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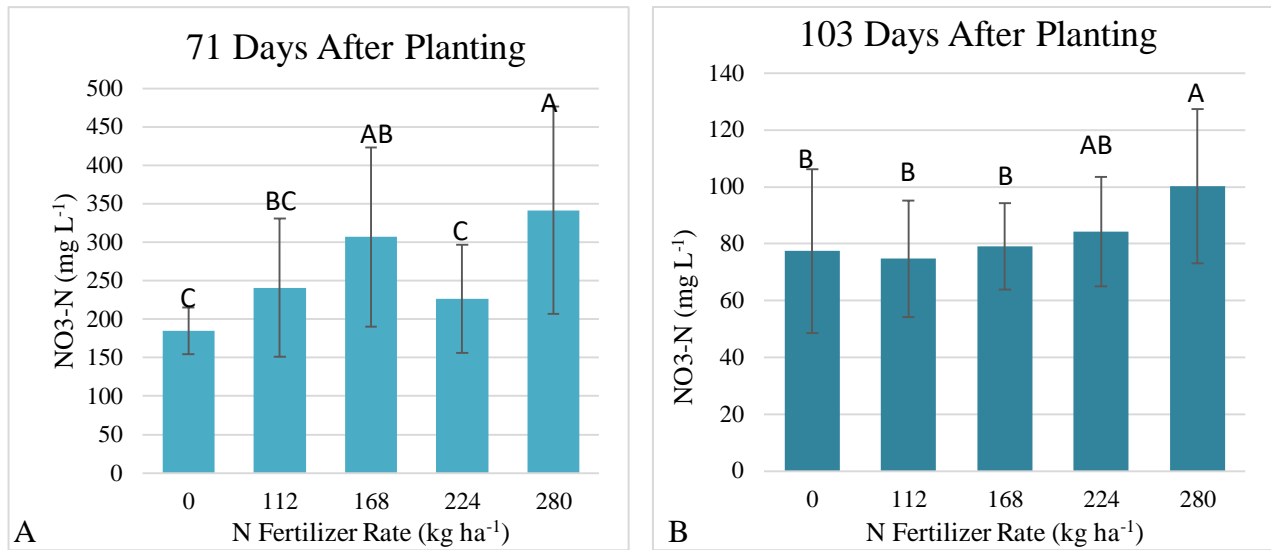
851 Sorghum sap NO<sub>3</sub>-N levels were significantly ( $p < 0.05$ ) increased by starter fertilizers  
852 early in the season (Figures 11A & 11B). Starter fertilizer increased sap NO<sub>3</sub>-N concentration  
853 17% at the 6-leaf stage (30 days after planting) compared to sorghum without starter fertilizer.  
854 There was not a difference between the in-furrow and 2x2 applications at this time. Similarly, at  
855 the 9-leaf stage (41 days after planting) there was an increase in NO<sub>3</sub>-N concentration for  
856 sorghum with starter fertilizer compared to sorghum without starter fertilizer. Sap NO<sub>3</sub>-N  
857 concentration was increased 9 to 19.5% compared to sorghum without starter fertilizer.



**Figure 11 A) NO<sub>3</sub>-N Concentration in Sorghum Midrib Sap 30 Days After Planting B) NO<sub>3</sub>-N Concentration in Sorghum Midrib Sap 41 Days After Planting.** Average NO<sub>3</sub>-N from the NIM when compared with starter fertilizer practices. Letters above bars indicate significance. Error bars represent one standard deviation.

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860 Following application of N fertilizer at 34 days after planting, NO<sub>3</sub>-N concentration  
 861 increased with increasing N fertilizer rates (Figures 12A & 12B). At 71 days after planting, NO<sub>3</sub>-  
 862 N concentration was greatest in sorghum receiving a total of 168 and 280 kg N ha<sup>-1</sup>. The lowest  
 863 NO<sub>3</sub>-N concentration was observed in the control (0 N) and N rate of 224 kg ha<sup>-1</sup>. There was a  
 864 46% increase of NO<sub>3</sub>-N in the 280 kg ha<sup>-1</sup> when compared to the control. Sap NO<sub>3</sub>-N  
 865 concentration at 103 days after planting was greatest where 280 kg N ha<sup>-1</sup> was applied, although  
 866 not significantly greater than 224 kg N ha<sup>-1</sup>. Despite lack of response for canopy reflectance  
 867 with starter fertilizer or increasing N fertilizer rates, differences were observed in sorghum sap  
 868 nitrate concentration. Early season differences were due to starter fertilizers and later season  
 869 differences were revealed following application of N fertilizer. The NIM shows promise and  
 870 potentially greater sensitivity for monitoring sorghum nitrogen status during the season.



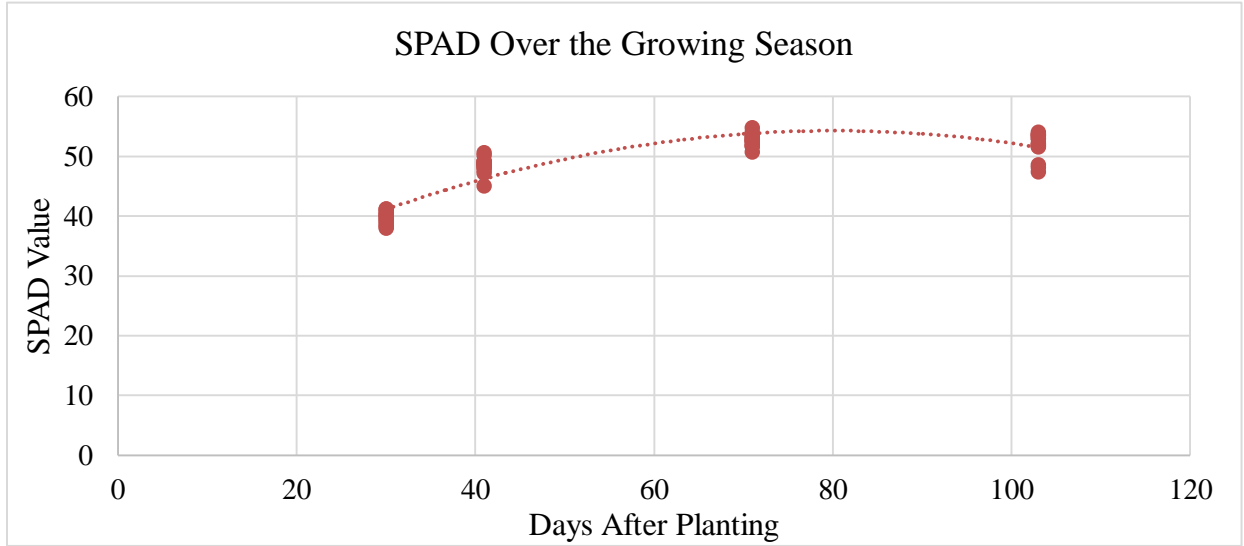
**Figure 12 A) NO<sub>3</sub>-N Concentration in Sorghum Midrib 71 Days After Planting B) NO<sub>3</sub>-N Concentration in Sorghum Midrib 103 Days After Planting.** Average NO<sub>3</sub>-N from the NIM when compared with increasing N rates. Letters above bars indicate significance at  $p < 0.05$ . Error bars represent one standard deviation.

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873 Chlorophyll Content in Sorghum

874 In conjunction with sap nitrate measurement, SPAD values were collected throughout the  
875 growing season (Figure 13). The mean SPAD value was 39 at 30 days after planting and peaked  
876 around 52 at 75 days after planting. Ma et al. (2005) found similar results in a N management  
877 corn study, where early seasons SPAD readings were 32 (V5) then increased to 65 by silking  
878 (R1). Values decreased at maturity.

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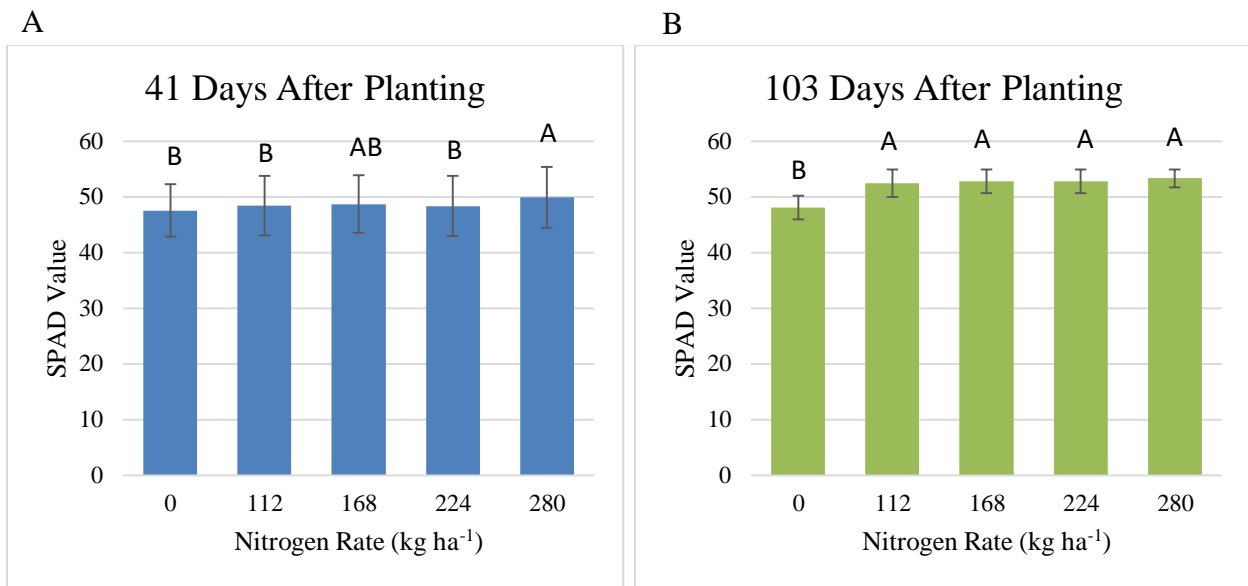
881 **Figure 13 SPAD Over the Growing Season**

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884 Starter fertilizer treatments had no affect on SPAD values throughout the season  
885 ( $p>0.05$ ). N fertilizer rates did have an affect on SPAD readings at two of the four collection  
886 dates. At 41 days after planting, application of 280 kg N ha<sup>-1</sup> had a greater SPAD value than 0  
887 and 112 kg N ha<sup>-1</sup> (Figure 14A). At 103 days after planting, all sorghum receiving N fertilizer  
888 had significantly higher SPAD values ( $p=0.0003$ ) than sorghum without N fertilizer (Figure  
889 14B). However, there was no difference of SPAD values between N fertilizer rates. In contrast,  
890 Ma et al. (2005) found a linear increase in SPAD readings with increasing N rates for corn in two  
891 years out of a three years study. Similar to the current study, Rambo et al. (2010b) found that  
892 SPAD values did not differ between N rates above 80 kg ha<sup>-1</sup> in a N management corn study.  
893 Comparing treatment affects at 41 to 103 days after planting, the ability of SPAD to detect  
894 differences in sorghum N status may be limited as time from N fertilizer application increases.

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897 **Figure 14 A) SPAD 41 Days After Planting B) SPAD 103 Days After Planting.** Average SPAD Value when  
 898 compared with increasing N rates. Letters above bars indicate significance at  $p < 0.05$ . Error bars represent standard  
 899 deviation.

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902 **Conclusions**

903 Despite the lack of N stress in both years of the study, variation in NDVI, chlorophyll  
 904 content, and sap NO<sub>3</sub>-N was detected for grain sorghum in response to fertilizer management  
 905 practices. CropScan NDVI and NIM did differentiate sorghum with starter fertilizer from  
 906 sorghum without starter fertilizer at early growth stages. SPAD and NIM were able to detect  
 907 differences in N fertilizer rates at later growth stages. NIM was the only instrument capable of  
 908 detecting both early season impact of starter fertilizer and later season affects from N fertilizer  
 909 rates.

910 NDVI has been used to monitor N status and predict yield of multiple crops over the  
 911 growing season (Foster et al., 2017; Ma et al., 1996; Moges et al., 2007; Rambo et al., 2010b;  
 912 Wang et al., 2016). CropScan was unable to detect differences in the N rates throughout the  
 913 study. However, the lack of N stress in both years of the study likely reduced the ability of

914 canopy level sensors to detect differences among N rates. Similar to previous studies, residual  
915 soil N and mineralization of N in soil likely contributed to significant plant available N pools and  
916 reduced sorghum response to N fertilizer.

917           Handheld sensors were also able to detect differences in the N rates and starter practices.  
918 The NIM was able to detect differences in  $\text{NO}_3\text{-N}$  concentration at all collection dates in  
919 response to fertilizer practices. Early in the season, the NIM measured differences in starter  
920 practices. Later in the season, NIM detected differences due to N fertilizer rates. The nitrate  
921 meter could be used to quickly obtain N status of the crop without time and expense required for  
922 laboratory results. While SPAD was not as sensitive as NIM, SPAD may be useful for detecting  
923 more severe levels of N stress.

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CHAPTER V  
CONCLUSIONS

Starter fertilizer practices have been studied extensively in many crops and production regions around the world, but there has been minimal research on how starter practices affect optimum N rate and timing, NUE, and yield in grain sorghum in Texas. Split applying N in grain sorghum could decrease N leaching, increase yield and increase NUE (Ameen et al., 2017; Buah et al., 2012; Mahama et al., 2014; Mahama et al., 2016; Maman et al., 2017; Tilman et al., 2002). Starter fertilizers that contain N could affect optimal timing for side-dress N application to grain sorghum. The timing of side-dress N application is critical for optimizing yield, increasing NUE, and decreasing environmental hazards (Bermudez and Mallarino, 2002; Cassman et al., 2002; Randall et al., 1985; Shrestha et al., 2010; Zotarelli et al., 2015). To assess in-season variation of crop N status with the ultimate goal of precision application and management of N fertilizer, canopy level reflectance has been evaluated using numerous spectral sensors for many different crops (Graeff and Claupein, 2003; Singh et al., 2017; Wang et al., 2017; Zhao et al., 2005). The ability of spectral sensors to identify N status and allow farmers to alter N management could result in increased grain yield and NUE in grain sorghum (Graeff and Claupein, 2003; Singh et al., 2017; Zhao et al., 2005).

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In the present study, starter fertilizer applications increased grain yield in one of the two years regardless of N rate or timing of side-dress N. For the N rate study in 2016, application of starter fertilizer as a 2x2 band increased yield >14% compared to grain sorghum without starter fertilizer. For the N timing study in 2017, in-furrow and 2x2 application of starter fertilizer increased grain yield by 5.5% compared to sorghum without starter fertilizer. Improvements of grain yield due to starter fertilizer applications were observed despite moderate levels of soil test

947 P ( $> 40 \text{ mg kg}^{-1}$ ) and the presence of substantial soil N. This suggests that applying small  
948 amounts of N and P near or with the sorghum seed may improve early season uptake of N and P  
949 and result in greater early season growth as reported previously for corn and sorghum (Gordon  
950 and Whitney, 1995; Mascagni and Boquet, 1996). In addition, starter fertilizers containing N and  
951 P have been reported to reduce the time to flowering (Mahama et al., 2014; Mahama et al.,  
952 2016). The combination of greater early season growth and reduced time to flowering can result  
953 in greater grain yield for sorghum, although yield improvements are not consistent.

954         Despite the potential for starter fertilizers to improve grain yield, starter fertilizers did not  
955 affect the optimum rate or timing of N fertilizer and did not consistently improve NUE. An  
956 optimum N fertilizer rate of  $168 \text{ kg N ha}^{-1}$  was observed in 2016 regardless of starter fertilizer  
957 practice. The latest date for application of side-dress N without grain yield reduction was 56 days  
958 after planting during 2016, 26 days greater than the current recommendation (30 days after  
959 planting) (Vanderlip, 1993). The optimum N fertilizer rate was less than  $110 \text{ kg N ha}^{-1}$  and there  
960 was no grain yield reduction with increasing delay of side-dress N application during 2017.  
961 Significant amounts of residual soil N and mineralization of previous corn crop residues and  
962 moderate levels of soil test P likely limited the ability to influence optimum N rates or timing of  
963 side-dress N with starter fertilizer applications.

964         The inability to develop significant N stress through variation of N rate or timing of side-  
965 dress N created a challenging environment for remotely sensing variation of sorghum N status.  
966 Handheld sensors demonstrated greater ability to detect variation in sorghum N status than  
967 sensors measuring canopy level reflectance. NDVI measured using CropScan was able to  
968 identify differences in starter fertilizer practices early in the growing season. Yet, CropScan was  
969 unable to detect differences in the increasing N rates in either year of the study. In contrast to



970 what other studies have found using CropScan NDVI with N management (Ma et al., 1996;  
971 Rambo et al., 2010a). In contrast, SPAD and NIM were able to identify differences in the N rates  
972 during 2017. SPAD detected variation in sorghum N rates at the two collection dates following  
973 side-dress N application. SPAD did not detect differences between starter fertilizer applications,  
974 despite increasing N and P amounts applied by contrasting starter practices. Yet, studies have  
975 shown SPAD to be a reliable source in detecting N status (Ma et al., 2005; Rambo et al., 2010b).  
976 In contrast, NIM was able to detect differences in  $\text{NO}_3 - \text{N}$  concentration from sorghum midrib  
977 sap at all four collection dates. Starter fertilizer practices increased midrib sap  $\text{NO}_3\text{-N}$   
978 concentration measured before side-dress N applications were made. Following side-dress N  
979 applications, midrib sap  $\text{NO}_3\text{-N}$  concentration increased as N fertilizer rates increased. NIM  
980 demonstrated the ability to differentiate fertilizer practices despite the lack of significant N stress  
981 or deficiency of soil test P. Low cost NIM's may be useful for detecting N stress in sorghum  
982 across a wide range of growth stages and production systems. Moreover, it may provide rapid  
983 feedback as proximal and remote sensors are evaluated for tools for N management in grain  
984 sorghum.

985 Starter fertilizers can increase early season growth, canopy reflectance and uptake of N in  
986 grain sorghum. While not consistent, starter fertilizers applied in-furrow or near the seed can  
987 increase grain sorghum yield potential. However, it is unclear whether starter fertilizers will  
988 affect the optimum rate or timing of N fertilizer in grain sorghum based on the current study.  
989 Significant residual soil N and mineralization of soil N precluded observation of N stress need to  
990 fully express treatment affects. Likewise, this reduced the ability to detect N stress in sorghum  
991 canopies using various reflectance sensors. Additional research under lower fertility and

992 minimal crop residues is needed to fully understand the impact of starter fertilizers on grain  
993 sorghum yield, optimum N rate, optimum N timing, and NUE in Texas.

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