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CORN GRAIN YIELD COMPONENTS AND NUTRIENT ACCUMULATION IN RESPONSE TO NITROGEN, PLANT DENSITY AND HYBRID

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CORN GRAIN YIELD COMPONENTS AND NUTRIENT ACCUMULATION IN RESPONSE TO NITROGEN, PLANT DENSITY AND HYBRID

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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by

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ABSTRACT OF THESIS

CORN GRAIN YIELD COMPONENTS AND NUTRIENT ACCUMULATION IN RESPONSE TO NITROGEN, PLANT DENSITY AND HYBRID

Modern maize hybrids exhibit higher yields, increased biomass production, stress tolerance and greater nitrogen (N) use efficiency. Increased biomass accumulation can influence nutrient uptake and lead to increased nutrient removal. Hybrids were tested at seeding rates (SR) of 74000 (low) and 148000 (supraoptimal) plants ha^{-1} and at N rates of 0 (deficient) and 390 (non-limiting) kg N ha^{-1}. Plants were sampled at V7, V14, R3, R5 and R6 and separated into vegetative and reproductive fractions for determination of dry matter and N accumulation. Grain yield was harvested at R6.

The high SR and high N treatment combination resulted in greatest biomass accumulation, crop growth rates, and N accumulation per hectare in both vegetative and grain tissues.

The high SR and high N combination maximized grain yield at 20.6 Mg ha^{-1}, essentially through an increase in kernels ha^{-1}. High SR decreased kernel weight, even with high N. At the higher plant densities resulting from the high SR, however, average utilization of available N was enhanced. The results have implications for improved management practices under high input systems and providing insight to growers who incorporate variable seed and N rates.

KEYWORDS: nitrogen rates, plant density, maize, nutrient uptake, grain yield, yield components.

Maria Julia Santoro
February 23, 2018

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1. Introduction

Maize (*Zea mays* L.) grain yields have exhibited steady increments since hybrids were introduced in the 1930s to 1940s in the U.S. (Duvick, 2005). Past growing trends resulted mainly from the application of simultaneous efforts; genetic gain achieved by plant breeding and improved management practices by producers (Tollenaar and Lee, 2002). Amongst the numerous management practices adopted by farmers to complement the genetic improvement of corn plants; the use of inorganic N fertilizers and higher plant populations were the most decisive factors. Such changes introduced the concept of high input systems, in which maize's strong dependence on plant density and N availability were emphasized (Egli, 2008: Sangoi, 2001). Modern corn hybrids exhibit lower lodging and higher tolerance to stresses. Consequently, they demand higher seeding rates than their predecessors to maximize yields (Reeves and Cox, 2013). Higher plant populations in maize can be achieved by decreasing row spacing and thus creating a more equidistant plant distribution within the plant community. As a result, the amount of solar radiation that is intercepted by the crop canopy can be increased earlier in the season (Andrade et al., 2002), particularly under high yielding environments.

By increasing plant densities in corn, one of the two major yield determining components; kernel number per unit area, is directly affected. This is a direct consequence of more ears per area being achieved when establishing higher seeding rates (Egli, 2015). Following this reasoning process, supraoptimal plant populations and ultra-high N rate treatments are thus expected to promote higher yields under irrigated production systems when compared to low plant densities, all other factors kept equal. Also, the higher levels of N fertilizer applied are expected to be sufficient for kernel weight development under increased plant population densities.

In view of a constant upward trend in maize plant populations in the last decades (Egli, 2015), it is vital to investigate physiological responses of corn in response to higher plant densities and how yield components might be influenced by the combined application of non-limiting N fertilizer. In this sense, evaluating crop biomass production and nutrient accumulation dynamics both per plant and at a community level will provide evidence to better understand grain nitrogen removal at harvest and the potential effect on kernel mass under contrasting nutritional scenarios.

1.1. Which key factors contributed to higher maize grain yields?

1.1.1. Higher Populations and Narrow Rows

Increased plant population density in maize production is the agronomic management tool that most contributed to maize grain yield increase in the past decades in the United States (Tokatlidis and Koutroubas, 2004; Egli, 2015). According to Egli (2015), the upward trend in plant density has been uninterrupted since the 1930s and has quadrupled during the so called ‘hybrid era’, increasing from 30,000 to approximately 75,000 plants ha^{-1} by the year 2000. However, after carefully examining the literature and comparing hybrids from different periods, the author also concluded that kernels per ear and number of ears per plant showed no increase as decades went by. Differences in maize grain yield are determined for the most part by kernel number per unit area (Maddonni et al. 2006; D’Andrea et al. 2008; Egli 2015; Andrade et al. 2000).

Consequently, the need to increase plant densities was imminent, turning maize into a highly population dependent crop. As a result, maximization of grain yield per unit area has been lately attributed almost exclusively to planting at greater densities (Raymond et al. 2009).

Improved modern hybrids exhibit higher tolerance to increasing seeding rates and other biotic stresses such as insect feeding and pathogen infection (Bender et al. 2013b). Also, much of the acquired ability to withstand and succeed at higher plant populations is due to greater efficiency to capture and use available resources such as water, solar radiation and nutrients (Boomsma et al. 2009; Raymond et al. 2009). All these advances found in today's commercial corn hybrids have been for the most part accomplished as a result of breeding efforts focusing on grain yield and improved morphophysiological characteristics. Because selection processes have been conducted in a wide range of production environments, the optimal plant population density to obtain maximum grain yield has increased in current production systems (Boomsma et al. 2009; Tokatlidis and Koutroubas, 2004).

In the field, planting higher population densities can be accomplished by an agronomic practice that enables a more equidistant spatial arrangement by reducing the distance between rows and at the same time increasing the space between plants within the row (Nielsen, 1988). This particular distribution of a plant community increases the amount of solar radiation that is intercepted by the canopy (Andrade et al. 2002; Porter et al. 1997; Barbieri et al. 2008). Greater light interception can be accomplished due to two major factors. The architecture of modern genotypes presents leaves that are more erect compared to plants from other hybrid eras (Bruns and Abbas, 2005). This allows for plant canopies to better withstand higher plant populations and at the same time permits solar light to enter through the canopy more efficiently, improving total crop light interception on an area basis (Barbieri et al. 2008; Bruns and Abbas, 2005). On the other hand, competition among plants in the same row is diminished using narrower rows (Porter et al. 1997). As a consequence of less plant-to-plant rivalry for available resources and increases in radiation interception, crop growth rate and thus biomass production are expected to improve (Andrade et al., 2002; Bullock et al., 1988; Shapiro and Wortmann, 2006; Farnham, 2001). In this sense, Bullock et al., (1988) came to the conclusion that growth rate during the early growth stages of the crop was higher when plants were distributed equally distant from each other (narrow rows are usually 38-cm wide) across the field as opposed to a conventional 76-cm row width arrangement.

A direct association has been established between the quantity of photosynthetically active radiation intercepted (IPAR, MJ m^{-2}) by the canopy and the crop growth rate (Andrade et al, 2002). Since the production of aerial dry matter is highly related to the amount of IPAR, (and hence to the crop growth rate) (Otegui, et al. 1995) there is sufficient research evidence demonstrating that planting corn in narrow rows increases grain yields (Porter et al, 1997; Van Roekel and Coulter, 2012; Crozier et al, 2014; Andrade et al. 2002; Shapiro and Wortmann, 2006; Bullock et al. 1988; Barbieri et al; 2008; Nielsen, 1988).

In a review conducted by Boomsma et al. (2009), plant population densities up to 104,000 plants ha^{-1} were tested in combination with nitrogen rates as high as 330 kg N ha^{-1}. The authors observed an increase in grain yield per unit area for each seeding rate when 165 kg N ha^{-1} were first applied. However, they found that the highest N rate treatment was required in order for the supraoptimal plant density to maximize grain yield per area. These findings would suggest that even higher seeding rates could be planted to additionally increase grain yields per area if nitrogen availability was not a limiting factor. Maddonni et al. (2006), when analyzing plant population densities as high as 120,000 plants ha^{-1} and a range of row spacings that went from 35 cm to 100 cm, reported highest grain yields when plant densities exceeded or equaled 90,000 plants ha^{-1}. In this study, 200 kg N ha^{-1} as urea were applied and furthermore considered a non-limiting N fertilizer rate.

On the other hand, research conducted by Ciampitti et al. (2013) using plant densities up to 104,000 plants ha^{-1} but a high N rate treatment of 224 kg N ha^{-1}, determined that grain yield response to nitrogen application (224 vs. 0 kg N ha^{-1}) was greater for medium (79,000 plants ha^{-1}) and high plant populations when compared against the low population density (54,000 plants ha^{-1}). In this case, differences in grain yield between medium and high plant densities could be potentially achieved under higher N rate treatments. These results are consistent with findings previously described by Boomsma et al. (2009) and Maddonni et al. (2006), in which they highlight the dependency of higher plant densities of maize on the amount of N applied in order to maximize grain yields.

Results from Raymond et al. (2009) agree with precedent examples. While testing plant densities up to 86,000 plants ha^{-1} applying an average of 257 kg N ha^{-1} under irrigated conditions, highest plant densities provided the greatest grain yields. The authors finally suggested that seeding rates recommended to producers should be increased based on grain yields showing no plateaus. In this regard, Egli (2015) explains how plant densities in corn production are destined to keep increasing in future years if kernels per ear and number of ears per plant remain unchanged. Interestingly, the 2016 National Corn Yield Contest reported highest grain yields that were obtained with 128,300 plants ha^{-1} under irrigation (National Corn Growers Association, 2016; Egli, 2015). Future populations are expected to be as high as 200,000 plants ha^{-1} if kernel number per plant and size remain constant (Egli, 2015).

Yield increases under narrow rows were associated with increases in solar radiation interception during the critical period for kernel set (Andrade et al., 2002; Barbieri et al., 2008). Barbieri et al. (2008) also attributed an increased N uptake to using narrow rows, an effect that can be explained partially by increased root length and a decline in plant to plant competition. The authors also analyzed the possibility of an increase in nitrogen use efficiency (NUE) as a result of improved N absorption during the earlier growth stages of the crop when planted under an equidistant distribution pattern.

In this sense, decreasing row spacing at high populations and guaranteeing sufficient N and water, would accelerate canopy closure by the plant community and increase the amount of light intercepted by the crop. Intensified management practices through the implementation of high input systems seem necessary to reach maximum yields in the future. Implementing 38-cm rows and ultra-high plant population densities in Kentucky can impact the way farmers produce corn in the next decades.

1.1.2. Nitrogen Fertilizer Use

In general terms, crop productivity has improved during the last hundred years due to a parallel increase in the use of nitrogen fertilizer (Massignam et al., 2009). Nitrogen is considered as one of the most grain yield limiting macronutrients in maize production

(Cirilo et al., 2009) and is highly associated with the photosynthetic process since up to 60% of N in leaves is linked to chloroplasts (Boomsma et al., 2009).

According to Smith et al. (2014), fertilizer used by U.S. farmers in the 1920s was approximately 6.6 million tons yr^{-1} and that amount increased to approximately 32.4 million tons yr^{-1} in the 1960s. Nitrogen fertilizer use on maize crops in the United States averaged 58 kg N ha^{-1} in 1964 and rose to 157 kg N ha^{-1} in 1985. Since that period, usage of nitrogen fertilizer has become stable at an average of 145 to 150 kg N ha^{-1} (Duvick, 2005). This is consistent with data presented by Smith et al. (2014) regarding Iowa maize N fertilizer usage. In 2016, U.S. corn producers applied N at an average of 163 kg N ha^{-1} (USDA-ERS, 2017).

According to Duvick (2005), the increment of plant population density in maize after World War II (1939-1945) followed a close trend with the rising amount of N fertilizer applied. Both higher plant populations and increased N fertilizer usage definitely shaped the constant increase experienced by corn grain yields in the past decades (Egli, 2008). This could be evidence that the even higher plant densities required in the future to sustain yield increases will therefore involve further intensified agronomic practices (e.g., higher N fertilizer rates, narrower rows, etc.; Egli, 2015). In this sense, to maximize yield under irrigated maize production, increasing plant densities may possibly demand higher applications of N fertilizer compared to current N rates being used.

In a review conducted by Boomsma et al. (2009), research including hybrids from the 1970s and 1990s found the latter to be more responsive to N applications. This greater response triggered an increased N fertilizer rate application amongst corn producers in the years that followed. Crozier et al. (2014) observed increased yields using narrow rows and sidedress N application compared to wider rows when evaluating nitrogen fertilizer response in different regions of North Carolina. Moreover, a 35% grain yield increase above the 0 N treatment (or control) took place when 224 kg N ha^{-1} were applied. However, yields plateaued at 179 kg N ha^{-1}, since no significant differences were observed between these two highest and consecutive N rates. The same was observed for yield components. Individual kernel mass experienced a 12% increase above control N treatments, but exhibited a maximum increase at 179 kg N ha^{-1}, as no significant differences were observed when the higher N rate was applied. On the other hand, kernel number components (rows ear^{-1} and kernels row^{-1}) plateaued at even lower N rates applied (134 kg N ha^{-1}). Similar results have been reported by Ciampitti et al. (2013), with grain yield per unit area improving as the N treatment was increased when averaged across plant densities and hybrids, but observing yields reaching maximum values at medium plant populations of 79,000 plants ha^{-1}.

This information contributes to the notion that greater N fertilizer rates might be required if future maize yields are to be maximized under uninterrupted increases in plant populations. This holds true principally since modern commercial corn hybrids present a higher potential for total biomass production. Increases in biomass yield are intensified by the combined effect of narrow rows and higher plant populations, which magnifies the demand for N (Shapiro and Wortmann, 2006). Consistent with this approach, Barbieri et al., (2008) observed higher total dry matter, grain yield and N accumulation with reduced row spacing and increased N rates (0 to 180 kg N ha^{-1}).

The intensification of input levels through management practices in high yielding environments might be determinant in the years to come for corn production. Moreover, the importance of evaluating the interactive effects of plant population densities and N rates among hybrids and their influence on grain yield and grain yield components seems vital.

1.1.3. Improved Nitrogen Use Efficiency in Modern Maize Hybrids

Modern corn hybrids, according to some researchers, are more efficient at taking up and using N (Ciampitti and Vyn, 2011; Crozier et al., 2014; Bender et al., 2013; Ciampitti and Vyn, 2012; Duvick 2005; Modhej et al., 2014; Below et al, 2007). According to Below et al. (2007) this could be a side effect resulting from genetic breeding programs focusing mainly on increasing grain yield throughout the years. Grain yield improvement has been achieved principally due to increased stress tolerance in plants, translating in better use of available N. Evaluation of NUE is important to better understand whether grain yield variations are due mainly to improved genetics, agronomic practices such as variable N rate levels and plant densities, or interactions between these parameters.

Modhej et al. (2014) observed across different N levels (ranging from 0 to 260 kg N ha^{-1}) that hybrids with greater yield also had greater NUE at every N rate tested. In their study, the newest hybrid had the highest yield and NUE. McCullough et al. (1994) reported similar results when comparing hybrids that spanned 30 years in Canada, where the newest hybrid had the greatest NUE, even at the lower N supply. They attributed the higher NUE to higher N uptake among other factors. Not all studies observed highest N uptake and use efficiency in the same hybrid. When analyzing N rates from 0 to >300 kg N ha^{-1} of 55 commercial hybrids grown at 79,000 plants ha^{-1} in Illinois, Below et al.

(2007) observed an average NUE of 21.6 kg grain kg N^{-1} ranging from 6 to 42 kg grain kg N^{-1}. However, no hybrid tested displayed highest efficiency in both N utilization and N uptake, suggesting these parameters can still be improved, either by modifying agronomic management techniques or further improving plants by breeding efforts.

Studies evaluating the effects of plant density and sidedress N rates on maize NUE in Indiana concluded that combining an intermediate N rate of 165 kg N ha^{-1} and the highest plant density tested (104,000 plants ha^{-1}) resulted in the highest increase in NUE. Nonetheless, the NUE of 26.3 kg grain kg applied N^{-1} obtained in this case did not correspond with maximum grain yield, which was reached when applying 330 kg N ha^{-1} (Ciampitti and Vyn, 2011). Ciampitti et al. (2012) confirmed this observation again and detected a minimal association between N uptake and NUE. These results could be implying that greater use of applied N by the crop is not always linked to highest grain yields. Therefore, research should focus on investigating the influence of plant density and N treatments on N uptake efficiency to better comprehend NUE of improved maize hybrids.

1.2. Yield Components

In cereal crops, grain yield is determined by the number of grains per unit area and by the weight of each grain (Borras and Otegui, 2001). Grain number per hectare in maize is a function of plants per hectare, ears per plant, rows per ear and kernels per row (Crozier et al., 2014).

The potential size of a corn ear is influenced by the number of rows per ear and the amount of kernels per row (ear length). Rows per ear is commonly determined between growth stages V6 and V12 (Crozier et al., 2014). Hybrid genetics greatly influence rows per ear, but environmental factors can affect row number (Abendroth et al., 2011; Nielsen, 2003). Nitrogen deficiency before V8 reduced row number per ear (Elmore and Abendroth, 2006; Subedi and Ma, 2005) and was irreversible (Subedi and Ma, 2005). These findings strongly highlight the impact stresses could impose on row number and kernel determination when taking place early in the growing season.

Kernels per row combined with rows per ear determines total kernels per ear. Potential kernel number per row begins to set at V6 to V7 and development continues until V15 or V16, approximately one week before R1 (Abendroth et al., 2011; Crozier et al., 2014). Each ear is estimated to have a maximum of 700 to 1000 potential ovules or kernels. This parameter depends on the hybrid's genetics but can be strongly affected by environmental stresses. Environmental conditions taking place during vegetative stages and silking (R1) will affect the potential kernels to be formed. Adverse environmental conditions happening during or after R1 might reduce number of kernels produced (Andrade et al., 1999; Elmore and Abendroth, 2006; Abendroth et al., 2011). Ear development can stop completely and lead to abortion of that ear if growing conditions are not favorable (Andrade et al., 1999). In consequence, ear length is expected to change more frequently from year to year as it is more dependent on each growing season's available resources than is the number of rows per ear.

Rows per ear and kernels per row increased 3 and 19 percent, respectively, when comparing 224 kg N ha^{-1} to 0 N and with plant densities up to 90,000 plants ha^{-1} (Crozier et al., 2014). However, no significant differences were observed for rows per ear and kernels per row between 134 and 224 kg N ha^{-1}.

Generally, 400 to 600 kernels per ear are expected at harvest under growing conditions without stress (Nielsen, 2003; Abendroth et al., 2011). The actual number of kernels per ear is a significant factor when analyzing potential grain yield in maize.

Kernel mass is the other major component affecting maize grain yield and is determined once potential kernel number is set. Seed mass begins development after ovary fertilization takes place.

1.2.1. Kernel Number

Seed number is considered the main yield component of cereals and other crops, and is highly dependent on genotype, growing conditions and agronomic management factors (Vega et al., 2001). In maize, grain yield increments are predominantly associated with higher final kernel number and high correlation coefficients have been found between seed number and yield (Echarte et al., 2013; Carcova et al., 2003). Seed number depends on the establishment of potential kernels and most important of all, seed set. Spikelets generated as potential kernels always exceed the actual number of kernels set by the plant. In this sense, some researchers have pointed out the importance of improving the survival of these potential structures rather than the importance of continued work to increase their number. Consequently, studies have been focused on investigating factors that may cause potential kernels to abort, decreasing harvestable kernels at maturity (Carcova et al., 2003; Cirilo and Andrade, 1994; Otegui, 1997). In this regard, Cirilo and Andrade (1994) reported no changes in potential number of spikelets generated per ear at flowering when testing different sowing dates in Argentina and attributed final kernel number per ear to differences in kernel abortion. Moreover, the authors associated the decrease in kernel set to reductions in crop growth rate after silking. Otegui (1997) reported barrenness when using 160,000 plants ha^{-1} and observed spikelet abortion in all uppermost ears after silking, at both 80,000 and 160,000 plants ha^{-1}, all other resources unrestricted.

In maize, kernel number susceptibility to stresses was found to be highest during the period bracketing silking. As a consequence, environmental conditions and the physiological status of the crop during this period become vital for kernel set (Andrade et al., 1999; Andrade et al., 2000). The number of kernels that are established is highly reliant on photosynthate supply and this could help explain the documented association that exists between kernel number and plant growth rate during the critical period for kernel set (Echarte et el., 2013; Andrade et al.,1999).

A review by Echarte et al. (2013) concentrated on understanding the different mechanisms involved in the generation of grain yield in modern versus older maize hybrids reported that newer genotypes set more kernels per unit plant growth rate during the critical period for kernel set, as compared to the older hybrids. The first reason proposed was that newer hybrids exhibit a lower plant growth rate threshold for kernel set during the period bracketing silking. A second explanation would suggest that newer hybrids exhibit a higher potential kernel number when the availability of resources per plant increases compared to older genotypes.

When analyzing genotypic yield differences among Argentinean maize hybrids in response to plant populations, Hernandez et al. (2014) found that kernel number per plant was significantly associated with accumulated ear biomass. Also, a high correlation was obtained between the accumulated ear biomass and plant growth rate. A possible explanation proposed by the authors describes how the establishment of kernels is subject to the accumulation of biomass in the ear around the period of anthesis in corn and also depends on how efficient the reproductive structure is in using this biomass to set kernels. Under the stress generated at high plant densities (160,000 plants ha^{-1}), the authors found that genotypes differed in the amount of barrenness exhibited (Echarte et al., 2013). In this way, some hybrids showed greater plant growth rates when seeded at high stand densities and differences were also detected in the base plant growth rate needed for ear biomass accumulation.

Another important parameter that can affect kernel number set during the period that goes from 10 days before until 15 days after anthesis is the interval existing between pollen shed and silk emergence, commonly known as the anthesis-silking interval (ASI). Silk emergence can experience a delay when maize is planted at higher densities, resulting in an increased ASI and thus a decrease in pollination. This parameter is associated with barrenness, and the longer ASI observed at higher plant populations resulted in increased barrenness (Edmeades et al., 2000). Unfavorable growing conditions around silking might increase kernel number loss by floral asynchrony, lack of pollen or reduced pollen viability and a decline in silk receptivity (Anderson et al., 2004; Bassetti and Westgate, 1994). Deficient nitrogen supply can also delay flowering and silk growth in maize. Grain yield can then be limited because of a negative impact on pollination. Research done by Lemcoff and Loomis (1994) concluded that N deficiency reduces the final number of kernels and attributed these results to a decline in distal silk emergence. Interesting is the fact that kernel abortion was found to be more density dependent.

A study conducted by D'Andrea et al. (2008) evaluated hybrid kernel number at very contrasting N rates (0 and 400 kg N ha^{-1}) and observed differences amongst genotypes in their kernel number per plant response to plant growth rate during the critical period for kernel set. Findings showed a more stable N concentration in the ear compared to that of the vegetative component, suggesting that N supply influenced biomass partitioning between vegetative and reproductive fractions and that the ear is a priority sink.

Previous research on kernel number per ear and per area has been conducted in the United States and other grain producing countries, but none have included ultra-high plant population densities combined with non-limiting N rates and irrigation in an effort to evaluate high- yielding hybrids in a high input system.

1.2.2. Kernel Weight

Kernel weight is the second component of grain yield. Given that sink size has been determined earlier in the season, kernel weight is then considered less influential but a more stable component (Borras and Otegui, 2001; Egli, 1998).

Differences in kernel growth rate during the effective grain filling period are under genetic control and are connected to the amount of endospermic cells generated during the *lag* phase. On the other hand, a stressful environment characterized by factors such as drought, limited assimilate availability and high temperatures can affect seed fill duration considerably and thus the final kernel size that is achieved (Egli, 1998; Borras and Otegui, 2001; Eichenberger et al., 2015).

At higher plant densities, Poneleit and Egli (1979) found that yield per plant basis was reduced by 20%, with 6% from decreased kernel weight and 14% as a result of a decline in kernel number. Thus, kernel number is the greater factor in determining per plant yield. Lamm and Trooien (2001) reported that when studying the effects of increased maize populations, the decrease observed in kernel weight was usually compensated by more kernels per area. Similarly, research conducted in Iowa investigating kernel filling changes when selecting for grain yield detected a reduction in final kernel mass at high plant densities in selected stands, concluding that increases in grain yield were mainly due to a different yield component (Eichenberger et al., 2015).

In general, preceding studies have detected lower kernel mass at higher maize plant densities. Also, increasing levels of N fertilizer in several research efforts have indicated that final kernel weight plateaued at intermediate N rates (Crozier et al., 2014; Ciampitti et al., 2013). Higher seeding rates also reduced N removal in the harvested grain and this may impact grain N concentrations (Bender et al., 2013; Ahmadi et al., 1993).

1.3. Nutrient Uptake and Accumulation

Nutrients are essential for plant growth. Specifically, in maize production, certain nutrients are required in greater quantities. Macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) are accumulated in higher amounts in plant tissues, hence their importance to crop nutrition (Bender et al., 2013b; Abendroth et al, 2011; Havlin et al., 2014). In addition, N and P partitioning to maize grain is significantly higher than that for K. Nutrient uptake and partitioning dynamics in maize are associated with biomass production and grain yield (Setiyono et al., 2010). Furthermore, variations in nutrient availability and plant density have a direct impact on both aboveground dry matter and yield. Subsequently, as maize seeding rates have increased, and as maize yields are expected to continue an upward trend into the future (Smith et al., 2014), research effort focused on nutrient uptake and removal of high input-yielding maize production systems is vital.

1.3.1. Nitrogen

Nutrient acquisition at the root surface is predominantly accomplished by mass- flow, diffusion and root interception processes (Barber, 1962; Bender et al., 2013b). In the case of N, approximately 79% of this macronutrient reaches the root surface by mass- flow. Mass- flow of soil nutrients to the roots greatly depends on water uptake by plants and the nutrient concentration in the soil solution (Havlin et al., 2014). In moist soil, this mechanism can provide the nutrient to the root surface more quickly than diffusion, which requires a concentration gradient in the soil solution.

Nitrogen can be present in the environment as one of several forms. However, only nitrate (NO_3^-) and ammonium (NH_4^+) are available for the plant to absorb (Jones et al., 2013; Havlin et al., 2014). Most plants exhibit improved growth when both NO_3^- and NH_4^+ are absorbed. Nevertheless, NO_3^- must be reduced to NH_4^+ before utilization by plants, a process that involves energy costs to the plant.

The sum of nutrient accumulation in each plant component (stem, leaf, grain, etc.) gives total plant nutrient uptake. Depending on the nutrient evaluated, the uptake pattern can vary throughout the growing season and is usually associated with specific vegetative and reproductive developmental stages (Abendroth et al., 2011; Bender et al., 2013a). Total N uptake can be described by an S-shaped response curve. Two main periods of N accumulation were identified by Karlen et al., (1988) at high maize yield levels; the first occurring at vegetative stages V12 through V18 as yield potential is being set, and a second period during grain filling while grain yield is determined. At VT/R1, when pollination occurs in maize, a lag phase is observed in which N uptake by the plant is minimal.

Bender et al. (2013b) confirmed that approximately two-thirds of N uptake takes place during vegetative growth stages. In this regard, maximum nutrient uptake rates were observed by Bender (2012) during the period bracketing V10 to V14, during which 8.8 kg N were assimilated per hectare per day. These results highlight the importance of N application and availability during maize vegetative stages. The author also reported that a total of 286 kg N ha^{-1} was required to produce a 12 Mg grain ha^{-1} yield, with 166 kg N ha^{-1} removed in the grain at maturity. Consequently, about 58% of total N accumulation was contained in the grain in this study. Abendroth et al., (2011), on the other hand, reported a 0.67 nitrogen harvest index at R6.

Nutrient mobility within the plant influences the amount of nutrient partitioning to the grain. Nitrogen is highly mobile once assimilated by the plant and can be remobilized to grain quickly during the grain filling period (Bender et al, 2013a). Nitrogen remobilization from vegetative to reproductive organs may lead to deficiency symptoms in older leaves, located lower on the plant. Yellowing and chlorosis usually appear first on these leaves, which, with extreme N deficiency, can undergo senescence. Necrotic leaf tips are observed first, followed by necrosis along the midrib (representative V-shaped pattern) until the whole leaf turns brown and dies. These symptoms result from the loss of leaf chloroplast proteins. Newer leaves located in the uppermost part of the plant remain green due to the mobility of N within the plant (Havlin et al., 2014).

1.3.2. Phosphorus and Potassium

Phosphorus and K are primarily delivered to the roots by diffusion, given the immobile nature of both these nutrients in the soil system. Approximately 93 and 80% of P and K uptake, respectively, results from diffusion (Bender et al., 2013b). Diffusion through the soil solution takes place due to the concentration gradient created by root nutrient absorption. The nutrient ion moves from high nutrient concentration areas to a lower concentration area (the root surface). Diffusion is a slower process than mass- flow (Havlin et al., 2014).

Similarly to N uptake, K accumulation takes place largely during vegetative growth. In contrast, a significant amount of P uptake occurs during maize grain filling (50% uptake by pollination). In this regard, past studies have reported that practically 90% of total K uptake in maize occurs before R2 and that the P uptake rate is continuous from V6 to maturity (Bender et al., 2013a). About 1.0 and 5.1 kg ha^{-1} of P and K, respectively, are accumulated daily by the crop between V10 and V14 (Bender, 2012). A total of 49.8 and 168.3 kg of P and K ha^{-1}, respectively, were required for a 12 Mg ha^{-1} maize yield. However, 39.8 and 55.6 kg of P and K ha^{-1} were removed with grain harvest. As such, P exhibits a comparatively higher harvest index, and approximately 80% of this element was partitioned to the grain, demonstrating the high plant mobility of this nutrient. Conversely, K exhibited a low harvest index (0.33), as indicated by previous research (Karlen et al., 1988; Bender et al., 2013a; Bender, 2012; Abendroth et al., 2011; Setiyono et al., 2010). That K tends to be stored in stalk and leaves during the vegetative period and to be absorbed in quantities above those required by the crop (luxury consumption) are possible explanations for the low partitioning of K towards grain. Potassium is also highly mobile within the plant, leading to greater variation in K accumulation amongst the various plant component parts (Abendroth et al., 2011).

Phosphorus and K uptake by plants interact with soil N availability. According to Havlin et al. (2014), N benefits P uptake by increasing root growth (mass) as well as soil P solubility and availability. Similarly, Johnston and Milford (2012) found that an increased supply in N led to higher stalk N and K accumulations, until R1.

Chapter 2

2. Materials and Methods

2.1. Study Design and Treatments

Field research was conducted at the University of Kentucky Research Farm (Spindletop Farm) in Lexington, Fayette County, Kentucky, (38˚01’47’’N, 84˚29’41’’W) in 2015 and 2016 (LEX2015 and LEX2016, respectively) on a Lowell-Bluegrass (fine, mixed, active, mesic Typic Hapludalfs) silt loam. In 2016, field research was conducted at a private farm in Hardin County near Glendale, Kentucky (37˚ 26’34.4” N, 85˚ 55’30.3W”) (HAR2016) on a Crider (fine-silty, mixed, active, mesic Typic Paleudalfs) silt loam (Table 3.1). At LEX2015 and LEX2016, four replications were planted for grain yield and three replications were planted for destructive samples. At HAR2016, four replications were planted for both yield and destructive harvest. Each plot for both grain harvest and destructive sampling was 12 38-cm rows by 8.2 m long. A 0.9 m border was placed between ends of each plot. Machine harvest occurred in rows 2, 3, 4 and 5 while destructive sampling occurred in rows 8, 9, 10 and 11. The remaining rows served as borders to ensure uniform light interception among all sampled rows.

Treatments were arranged in a randomized complete block design arranged as a split-split-plot, containing three main treatment factors. Two N rates (0 kg N ha^{-1} [Zero N] and 390 kg N ha^{-1} [High N]) were assigned to the main plot, two maize hybrids (AgriGold A6499 and A6517; 112 days (d) and 113 d comparative relative maturity, respectively) as subplots, and two seeding rates (SR) as sub-subplots (Low, at 74,000 plants ha^{-1} and High, at 148,000 plants ha^{-1}). The same two hybrids were planted in all environments, but in 2016 four maize hybrids were added, both at LEX2016 and HAR2016. Dekalb DKC62-78RIB and DKC67-72RIB, with 112 d and 117 d relative maturities, respectively, and Pioneer P0339AM and P2089AM, with 103 d and 120 d relative maturities, respectively.

2.2. Planting

Corn seeds were planted 8 May, 26 April and 18 April at LEX2015, LEX2016 and HAR2016, respectively, with no prior tillage (no-tillage) following soybean (*Glycine max* L. (Merr.), a common rotation in Kentucky. These planting dates are optimal planting for central Kentucky, falling between April 15 and May 15 (Bitzer, 2001).

A Wintersteiger pneumatic planter (Salt Lake City, UT) with a slotted disc system and cone seed delivery, attached to a Case IH Puma 130 (Basildon, England) using a GPS RTK guidance system (Trimble; Sunnyvale, California), was used to establish the studies. To accommodate the extremely high seed population, seeds were placed into 38-cm rows at a targeted depth of 5 cm.

Stands were assessed at growth stage V3 (Abendroth et al., 2011) along the two center rows of each plot. On average, final plant populations were 10% less than targeted seeding rates. Uniformity of final stands at each seeding rate was successfully achieved in all environments.

2.3. Nitrogen Application

The objective of the chosen N rates (0 and 390 kg N ha^{-1}) was to cause limiting and non-limiting N conditions. Nitrogen fertilizer as urea ($CO(NH_2)_2$), grade 46-0-0, was applied broadcast at V3 in a single dose. Since the soils were well-drained, University of

Kentucky guidelines allow for a single application (AGR-1, 2015). However, the rate of 390 kg N ha^{-1} is considerably in excess of recommendation (196 to 224 kg N ha^{-1}). Surface applications of urea are subject to ammonia (NH_3) volatilization, but rainfall events occurring after application, at all three environments, likely reduced volatilization losses (Jones et al., 2013 and Murdock, 2001). Soil pH and cation exchange capacity values for these soils likely further decreased risks of volatilization (Murdock, 2001; Jones et al., 2013 and Stewart, 2008).

2.4. Management

In order to avoid moisture stress, irrigation at LEX2015 and LEX2016 was delivered via drip tape and by center pivot in HAR2016. Weed control each growing season in Lexington started with a pre-plant burn down herbicide application which included potassium salt of glyphosate: N- (phosphonometyl) glycine (approximately 1150 mL ai ha^{-1}). Three weeks after planting LEX2015, atrazine (494 mL ai ha^{-1}) and topramezone: (3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylphenyl) -(5-hydroxyl-1methyl-1H-pyrazol-4-yl) methanone (0.614 mL ai ha^{-1}), dimethenamid-P: (S)-2-chloro-N-[(1-methyl-2-methoxy) ethyl]- N-(2,4-dimethyl-thien-3-yl)-acetamide (30.8 mL ai ha-1) and glyphosate were applied.

At LEX2016, three weeks after planting, the herbicide application consisted of glyphosate (2267 mL ai ha^{-1}), followed by an application of S-metolachlor: (RS)-2-Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(1-methoxypropan-2-yl) acetamide (277 mL ai ha^{-1}), atrazine (271 mL ai ha^{-1}) and mesotrione: 2-[4-(Methylsulfonyl)-2-nitrobenzoyl] cyclohexane-1,3-dione (4.37 mL ai ha^{-1}) four weeks after planting.

At HAR2016, glyphosate (641 mL ai ha^{-1}) was applied eight weeks after planting as a directed spray and again as a directed spray at the VT/R1 stage, which corresponded to ten wk after planting. Weeds were not competitive with corn at any environment.

No insect or disease pressure justified foliar insecticide or fungicide treatments. However, some grey leaf spot (caused by *Cercospora zeae-maydis*) was observed at HAR2016 towards the end of the growing season (R5 growth stage). By then, a fungicide application would not have protected yield.

2.5. Climate

Data provided by Midwestern Regional Climate Center's stations throughout the state of Kentucky were used to obtain climate records for the different environments under study. For LEX2015 and LEX2016, the Lexington Bluegrass Airport station (USW00093820) was used. The Hodgenville-Lincoln Kentucky station (USC00153929) was used for HAR2016. This source of information was preferred primarily because a complete set of data for temperature and precipitation was provided and the stations were close to the study sites.

Daily, monthly and historic averages were used to describe climate trends. Growing degree days (GDDs) were computed using a base temperature of 10˚C as defined by Loecke et al. (2004):

$$GDD = [(T_{max} + T_{min})/2] - T_B,$$

where T_{max} is the daily maximum air temperature, T_{min} is the daily minimum air temperature, and T_B is equal to 10˚C for corn because 10˚C represents the base temperature required physiologically by the crop to guarantee growth.

2.6. Soil Sampling for Nitrogen

At least 15 soil cores for pH, CEC and nutrient analysis were taken pre-plant to a 10-cm depth and mixed together to create a composite sample for each environment (Murdock, 2011). These soil samples were sent to the University of Kentucky Regulatory Services Soil Testing Laboratory for analysis.

Soil samples for NO_3^- analysis were collected pre-plant at the 0 to 15 and 15 to 30 cm depth increments. Samples were left to air dry for a week and were crushed using a 2 mm sieve.

Determination of soil nitrate and ammonium (NO_3–N and NH_4^+-N) was done at the University of Kentucky Soil and Plant Analysis Research Laboratory. Approximately 10 g soil from each sample were added to a plastic centrifuge tube. Subsequently, 25 mL of a 1M potassium chloride (KCl) solution was added to each tube. The tubes were later placed on a shaker for 1 h and then left to settle for 1 h. Using a pipette, 1 mL of extract solution was removed from each tube, placed into a cluster tube, and the cluster tubes were then placed in a centrifuge for 27 minutes at 3700 revolutions per minute. Extracts were run on a microplate reader for NO_3–N and NH_4^+-N determination using known standards that were prepared in 1M KCl to match the matrix of the samples.

The amount of total inorganic N in the soils was calculated by adding the NO_3–N and NH_4^+-N values obtained from the analyses (Table 4.1).

Soil samples from both 2016 environments were immediately placed in refrigerated storage but were accidentally removed early. Consequently, total N values from 2016 are considerably higher when compared to 2015 due to mineralization and nitrification processes that were favored by increased temperature and time prior to extraction.

2.7. Combine Harvest

Grain harvest was completed using a Wintersteiger plot combine (Wintersteiger AG, Ried, Austria) equipped with a Harvest Master weight bucket system with moisture sensor (Juniper Systems, UT). At all environments, the center four rows of each plot (12.5 m^2) were harvested to obtain grain weight.

2.8. Plot Ear Harvest

At maturity (growth stage R6), plants were hand harvested from each of the plots designated for destructive sampling in LEX2015. Plants and ears from 3 m of the second row were collected and counted. Whole plant biomass was weighed and then ears were separated from the fodder (stem and leaf) portion and weighed. At LEX2016, plants and ears were collected from 1,5 m of row. At HAR2016, ears were collected from 1,5 m of row.

Ear samples were dried at 60 ˚C to a constant weight and weighed dry biomass determined.

Fodder (stem and leaf), husk and cob sample processing procedures will be fully described when addressing plant biomass and nutrient uptake measurements.

2.9. Post - Harvest Measurements

2.9.1. Kernel Number and Kernel Mass

Once dried, three maize ears were subsampled for kernel row number and kernel number per row. An electric sheller separated grain from cob. Kernel weight was determined by selecting 250 kernels with an electronic seed counter, weighing these, and adjusting the mass to a 1000 kernel basis (Crozier et al., 2014).

2.9.2. Ear Length and Tip-Back Length

Ear length and tip-back were measured on the three maize ears at LEX2016 and HAR2016. The term “tip-back” generally is used to describe the lack of kernels present at the terminal end of the cob (the tip). Field stress conditions during the critical period for kernel set (± 15 days around R1), such as N deficiency and/or high plant population, could cause greater tip-back (Nafziger, 2016). Tip-back length was measured as the distance from the last visible kernel to the tip of each ear.

2.9.3. Harvest Index

Harvest index for each plot, in all environments, was calculated as the weight ratio of grain dry matter to total aboveground plant dry matter (Ciampitti and Vyn, 2011).

2.10. Aboveground Plant Biomass and Nutrient Uptake

At LEX2015 and LEX2016, five consecutive plants from either the third or fourth row were collected from each plot designated for the destructive samplings. Plants were cut at ground level. Plant density was well represented for each treatment.

Whole plant harvest at LEX2015 and LEX2016 was conducted at V7, V14, R3, R5 and R6. Once the five plants were weighed, separated into vegetative (stem and leaf) and reproductive (ears and husks) fractions and fodder parts were coarsely subdivided to obtain a subsample, all was dried to a constant moisture at 60 ˚C (Ciampitti and Vyn, 2011). At V7 and V14, vegetative material consisted of stems and leaves. At R3, plant material was separated into stem plus leaves and a reproductive fraction comprised of kernels plus cob and husks. At R5 and R6, grain was separated from the cob and the reproductive fraction included grain, cob and husk as separate components. All fractions, at all growth stages, were weighed for dry biomass. In order to obtain the amount of each nutrient (N, P, K) that each fraction contained, the nutrient concentration in that fraction was multiplied by the dry matter weight.

For LEX2015 and LEX2016, plant N, P, and K uptake per hectare were calculated, taking under consideration individual tissue dry matter weights, nutrient concentrations, and plant population density. Likewise, the same procedure was conducted for cob, husk and grain nutrient uptake (Crozier et al. 2014).

2.11. Nutrient Analyses

To determine nutrient content in plant tissue, dried vegetative and reproductive samples were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Fodder and grain samples were passed through a Udy Mill (Fort Collins, CO) equipped with a 0.5- mm sieve.

Nitrogen and P analyses for fodder and grain were conducted using a micro- Kjeldahl digestion method. To prepare the samples for the digestion, 100 mg of dried material was weighed into 25x200 Pyrex glass tubes. The digestion procedure consisted of adding concentrated sulfuric acid and potassium sulfate to samples and heating on a block digester (Bradstreet, 1965).

Next, simultaneous colorimetric determination of total N and P was done using a dual Technicon System II Auto-analyzer. The method used for N was developed by Chaney and Marbach (1962) and a procedure based on the method of Fiske and Subbarow (1925) was used for P. In both cases, standards were prepared for calibration in order to minimize errors.

Potassium grain analyses were conducted weighting 250 mg of sample material into 50 ml flasks. Samples were then placed in a muffle furnace (Thermolyne 30400) and 2 mL of concentrated HCl (12.1 N) were added. After dilution of the samples by adding 23 mL of water, a final 1N HCl solution was obtained. Next, dilutions (1:20) and standards were made and samples run on an atomic absorption spectrometer (SpectrAA 50B).

Phosphorus and potassium values are reported as P and K, respectively.

2.12. Nitrogen Deficiency Rating

For LEX2015, nitrogen deficiency ratings were recorded at R5. For LEX2016, ratings were done at R1, R3 and R5 and at R3 and R5 for HAR2016.

Nitrogen deficiency can be observed in the field mainly in the lower leaves of the crop canopy, visible as a “V” shaped yellowing coloration (Murdock, 2001). This symptom starts at the leaf tip and travels along the midrib towards the stem on the plant. Visual symptoms were evaluated on five tagged plants located in the fourth row of each plot designated for non-destructive sampling. The dominant ear leaf was designated as “0” and each leaf below was given a negative number (i.e. -1 for the first leaf below the dominant ear leaf) while leaves above the dominant ear leaf were denoted with positive values.

2.13. Nitrogen Indices

2.13.1. Nitrogen Harvest Index (NHI)

The NHI (Sinclair, 1998), is used to help explain nitrogen partitioning in the plant and can be expressed as the ratio between nitrogen accumulated in grain and the total N accumulated at plant maturity (Bender et al., 2013; Cirilo et al., 2009; Boomsma et al., 2009). This parameter was determined for LEX2015 and 2016.

2.13.2. Nitrogen Internal Efficiency (NIE)

The NIE was calculated for LEX2015 and LEX2016 as the portion of the grain yield response attributable to N uptake. NIE was calculated as follows (Ciampitti and Vyn, 2011):

$$NIE = (GY_{fert.} - GY_{unfert.}) / (Nupt_{fert.} - Nupt_{unfert.}),$$

where $GY_{fert.}$ is the grain yield (15.5% moisture basis) of a plot fertilized with 390N and $GY_{unfert.}$ refers to the grain yield of a plot receiving 0N. $Nupt_{fert.}$ is N uptake corresponding to the fertilized plot and $Nupt_{unfert.}$ is N uptake for the unfertilized plot.

2.14. Statistical Data Analyses

2.14.1. Grain Yield and Yield Components

The analysis of variance (ANOVA) was completed using a linear additive model that tested environment, replication (block), N rate, hybrid, seeding rate and all interactions using a PROC GLM statement in SAS 9.4 (SAS Institute Inc., 2013). The treatment factors (N rate, hybrid and SR) and the environments (site- year) were considered fixed in the analysis, and replications were considered random. Each environment contains intrinsic characteristics such as soil type, climate, etc., which makes possible an evaluation of the effect of environment on crop response to the applied treatments. When treatment effects were significant at *P* ≤ 0.10, the least-squares means (LSMEANS) were tested.

The analysis first included all three environments (LEX2015, LEX2016 and HAR2016), but included only the two hybrids common to all environments (A6499 and A6517). Since environment interactions with main factors were significant (p ≤ 0.10), each environment was analyzed separately. All hybrids in each environment were analyzed. The same linear model was used, without environment as a source of variation.

2.14.2. Nutrient Uptake

A repeated measures statement was used to analyze nutrient uptake since biomass harvests and measurements were taken with time, though at different growth stages. To test the ANOVA (α ≤ 0.10), the linear additive model included environment, time (growth stage), replication (block), N rate, hybrid, seeding rate and all the interactions using a PROC MIXED statement in SAS 9.4 (SAS Institute Inc., 2013). When treatment effects were significant, the least-squares means (LSMEANS) were tested using the PROC GLIMMIX statement.

Whole-plant biomass was sampled in both LEX2015 and LEX2016. These measurements included stem and leaf, grain, husk and cob fractions. The stem and leaf nutrient uptake analysis was conducted over all growth stages. In the case of grain accumulation, the analysis included only R5 and R6 stages. Since at R3 grain plus cob were analyzed together for nutrient uptake (grains were not separated from the cob), this stage was statistically analyzed by itself (Ciampitti et al., 2013). As for husk, the analysis was performed for the R5 stage in 2015 and a repeated measures statement was used for

2016, where R3, R5 and R6 stages were included in the analysis. Similarly, cob nutrient uptake was analyzed for R5 in 2015 and for R5 and R6 in 2016.

At HAR2016, on the other hand, only grain samples were harvested at R6 and therefore just grain nutrient removal analysis was conducted for this environment. When applicable, the nutrient accumulation analysis was first done comparing environments, including only the two hybrids that all environments have in common (A6499 and A6517). If environment or environment by main factor interactions were significant ($p \leq 0.10$), each environment was then analyzed separately. In this case, the four hybrids (DKC62-78RIB, DKC67-72RIB, P0339AM and P2089AM) added to the studies in 2016 were included in the statistical analysis. The same linear model was used, without environment as a source of variation. If, on the contrary, no significant differences were observed between environments, then statistical analyses were performed across environments, only including hybrids A6499 and A6517. To test the other four hybrids, a different statistical analysis was conducted for that environment including all hybrids ($n = 6$).

2.14.3. Crop Growth Rate

An analysis of covariance was completed using a PROC MIXED statement in SAS 9.4 (SAS Institute Inc., 2013). Crop growth rate (CGR) was determined as the slope of the linear regression between aboveground biomass and days after planting (DAP).

Chapter 3

3. Grain Yield and Yield Components

3.1. Objectives

Corn grain yield is greatly associated with the level of N that is available for the crop, as well as plant population. In this study, the N treatments were to cause N to be either limiting or non-limiting. In addition, plant population treatments were such that plant density was either suboptimal (Low SR) or supraoptimal (High SR). The following hypotheses were formulated:

a. Grain yield per hectare will be higher for all hybrids when combining the High N rate and the High SR.
b. High SR will increase kernel number per hectare and decrease kernel number per ear, however, High N will offset the effect of High SR.
c. Kernel weight will be greater with the High N rate, and will decrease marginally with the High SR as compared to the Low SR.

3.2. Results

3.2.1. Weather Conditions

The LEX2015 monthly temperatures were near average in April, warmer than average in May, near average in June and July, and below average for August. Lexington rainfall was above average in April, June and July and below average the other months of the growing season (Figure 3.1A, 3.1B). Growing degree days (˚C) for LEX2015 from April to August numbered 1656.

The LEX2016 temperatures were below average for May and above average for all other months of the growing season. The LEX2016 rainfall was above average in May and August and below average for all other months of the growing season.

The HAR2016 temperatures were below average for May and above average for all other months. The HAR2016 rainfall was above average in April, May, July and August (Figure 3.1C, 3.1D). June was below average for rainfall and there was a gap in meaningful rain for the last two weeks of June, disrupted by about 13 cm occurring over a week (Midwestern Regional Climate Center, 2017).

Growing degree days (C˚) for LEX2016 and HAR2016 from April to August were 1771 and 1725 C˚, respectively.

3.2.2. Grain Yield

There were numerous and inconsistent interactions between N rate and seeding rate effects on yield and yield components. The two hybrids were consistent across all three environments and the three-way interactions of N rate, seeding rate and hybrid and environment, N rate and seeding rate were significant ($p = 0.0104$ and $p = 0.0186$, respectively; Table A.1, Appendix). Because of these interactions, all six hybrids were analyzed for both 2016 environments, where the three-way interactions of N rate, seeding rate and hybrid ($p = 0.0079$) and environment, N rate and seeding rate ($p = 0$ .0928) were significant (Table A.2, Appendix). Since these interactions were significant, N and seeding rate effects on grain yield were analyzed by environment.

At LEX2015, mean yields were 9.8 and 17.5 mg ha^{-1} for the Zero and High N rates, respectively. Mean yields for the Zero and High N rates at LEX2016 were 7.2 and 18.0 Mg ha^{-1} and 6.9 and 12.5 Mg ha^{-1} at HAR2016, respectively.

For LEX2015 a significant interaction of N rate by seeding rate by hybrid occurred ($p = 0.0781$) (Table 3.2). The High N rate increased grain yield in all seeding rate by hybrid comparisons (Table 3.3). At the Zero N rate, the High SR reduced A6499 yield. The converse occurred at the High N rate where High SR increased yields (Table 3.3).

At LEX2016, a significant three-way N rate by seeding rate by hybrid interaction occurred ($p = 0.0576$). The High N rate increased yield of all hybrid by seeding rate combinations (Table 3.3).

Grain yield at HAR2016 was affected by two-way interactions, including N rate by seeding rate ($p < 0.0001$) and hybrid by seeding rate ($p = 0.0027$; Table 3.2). When N was limiting, High SR reduced yield by 34%, but, when N was non-limiting, High SR increased yield by 15%. The High SR increased grain yield for two hybrids (A6517, DKC62-78), decreased yield for hybrid P20892 and had no effect on the other three hybrids when averaged across N rate (Table 3.3).

As indicated earlier for the other two environments, grain yield increased with the High N rate treatment (Table 3.3). The main effect of seeding rate was not significant ($p = 0.2233$), but as previously observed in LEX2016, the High SR resulted in increased grain yield at the High N rate (significant N rate by SR interaction, $p < 0.0001$: Tables 3.2 and 3.3). Alternatively, the High SR reduced yields at the Zero N rate.

3.2.3. Grain Yield Components

3.2.3.1. Kernel Number

When analyzing the two hybrids across all three environments, all parameters involved in the determination of the kernel number component (rows ear^{-1}, kernels row^{-1}, kernels ear^{-1} and kernels ha^{-1}) exhibited a significant environment by hybrid two-way interaction (Table A.1). When analyzing the six hybrids across the two 2016 environments, other interactions occurred, usually involving environment by hybrid (kernels ha^{-1}, $p = 0.0554$; and kernels row^{-1}, $p = 0.0055$) or environment by N rate (kernels ha^{-1}, $p = 0.0008$; rows ear^{-1}, $p=0.0434$; and kernels row^{-1}, $p = 0.0260$) or environment by seed rate (kernels ha^{-1}, $p = 0.0515$; and rows ear^{-1}, $p = 0.0270$) (Table A.2).

Because of these interactions, each environment was analyzed separately for yield components.

The High N rate increased A6517 kernels ha^{-1} by 89 % at LEX2015. At the Zero N rate, SR had no effect on kernels ha^{-1}, while at the High N, the High SR increased kernels ha^{-1} by 20%, on average (Table 3.3). At HAR2016, the High N rate increased kernels ha^{-1} in 5 out of 6 hybrids at the High SR.

When differences were significant, the High SR increased kernels ha^{-1} at the High N rate in all three environments. In two environments, the High SR at the Zero N rate reduced kernels ha^{-1} (Table A.4). These results suggest that N rate was more influential on kernels ha^{-1} than seeding rate, for the kernel number parameters evaluated.

Mean kernels ear^{-1} for the Zero and High N rates were 473 and 627, 425 and 691, and 449 and 603 for LEX2015, LEX2016 and HAR2016, respectively. The High N rate increased kernels ear^{-1} for 21 of 28 hybrid by seeding rate combinations (across all three environments). In the remaining seven combinations, kernels ear^{-1} was numerically higher with the High N rate (Table A.3). The High SR reduced kernels ear^{-1} in 19 combinations. Kernels ear^{-1} was numerically lower for the High SR in the remaining comparisons (Table A.3). The highest kernels ear^{-1} values were observed at LEX2016 for P2089, High N rate, Low SR (895) and A6517, High N rate, Low SR (855).

Mean kernels row^{-1} for the Zero and High N rate treatments were 31.6 and 39.3, 28.2 and 40.6, and 29.9 and 37.7 for LEX2015, LEX2016 and HAR2016, respectively. The High N rate increased kernels row^{-1} in 19 hybrid by seeding rate combinations, with higher numerical kernels row^{-1} values for the remaining 9 combinations (Table A.3). The High SR reduced kernels row^{-1} in 20 hybrid by N rate combinations, with lower numerical kernels row^{-1} values for the remaining combinations (Table A.4).

Mean kernel rows ear^{-1} for the Zero and High N rate treatments were 15.0 and 15.8, 14.6 and 16.8 and 14.9 and 16.0 for LEX2015, LEX2016 and HAR2016, respectively. The high N rate increased kernel rows ear^{-1} in only nine hybrid by seeding rate combinations. The High SR decreased kernel rows ear^{-1} in only eight hybrid by N rate combinations (Table A.3).

In 2016, both at Lexington and Hardin, ear length decreased as actual plant population increased, regardless the N rate (Figure 3.2A, 3.2B). In both environments, the High N rate increased ear length. At LEX2016, ear length regression slopes declined equally as plant populations increased (Figure 3.2A). However, at HAR2016, ear length declined more rapidly at the Zero N rate as plant population increased (Figure 3.2B).

Predicted ear lengths both at LEX2016 and HAR2016 for significant regressions were longer at 74000 plants ha^{-1} target population when analyzing each hybrid across N rate treatments. Ear length ranged from 17.8 to 20.2 at LEX2016 and from 16.8 to 20.5 cm at HAR2016 (Table 3.4). At high target populations, predicted ear length values at LEX2016 and HAR2016 ranged from 12.4 to 14.6 cm and 11.3 to 15.1 cm, respectively.

Hybrid P2089AM produced the longest predicted ear length at both LEX2016 and HAR2016 for the Low population. P2089AM also had the longest ear at 148,000 plants ha^{-1} at HAR2016.

Actual plant population had no significant effect on tip-back, at either N rate, in 2016 (Figure 3.2C, Figure 3.2D). At LEX2016, actual plant population density did not influence tip-back. At HAR2016, hybrids A6499, DKC67-72, P0339AM, and P2089AM all had greater tip-back as plant density increased (Table 3.5). Overall, predicted tip-back increased 0.4 cm on average across hybrids as target plant densities increased from 74,000 to 148,000 plants ha^{-1}.

3.2.3.2. Kernel Weight

Average thousand kernel weights for the Zero and High N rates were 244 and 328 g, 258 and 312 g, and 278 and 288 g for LEX2015, LEX2016 and HAR2016, respectively (Table 3.3). On average, the High N rate increased kernel mass by 35% at LEX2015, as compared to the Zero N rate. At LEX2016 and for all hybrids, the High N rate and High SR treatment combination decreased kernel mass by 27%, on average, as compared to the Low SR. On average, the High N rate and High SR decreased kernel mass by 19% at HAR2016. In addition, High SR decreased kernel mass for five out of the six hybrids when averaged across N rates (Table 3.3). The High N rate did not overcome the High SR effect on reduced kernel mass. Thus, N availability does not appear to be the factor limiting seed size at high plant densities.

3.2.4. Biomass

Stem and leaf (aboveground vegetative material) biomass was evaluated over time with biomass harvests at V7, V14, R3, R5 and R6. Vegetative biomass was significantly affected by environment ($p < 0.0001$) when comparing LEX2015 and LEX2016 (Table 3.6). Therefore, each environment was independently statistically evaluated.

At LEX2015, leaf and stem biomass reached maximum levels at R3 and maintained those dry matter yields through R6 (Tables 3.11 and 3.12). The High N rate increased stem and leaf biomass from V14 through R6, but not at V7 ($p = 0.5113$; Table 3.11). Hybrid leaf and stem biomass responded differently to seeding rate. The High SR increased A6499 leaf and stem biomass at V7 and V14, but not at any reproductive growth stage (Table 3.12). The High SR had no effect on A6517 leaf and stem biomass.

At LEX2016, statistical analysis of stem and leaf biomass found three way interactions between N rate, hybrid and sampling time ($p = 0.0088$) and N rate, seeding rate and sampling time ($p < 0.0001$; Table 3.6). In most N and seeding rate treatment combinations, vegetative biomass was highest at the R3 stage, followed by the R5 stage, in most cases (data not shown). For both N rates, from growth stages V7 to R5, the High SR increased stem and leaf dry matter (Table 3.13). The High N rate increased stem and leaf biomass at all growth stages and seeding rates, except for the Low SR at V7 (Table 3.13). Thus, N supply at early developmental stages did not affect stem and leaf biomass at low plant densities. Stem and leaf biomass was maximized at R3 for all hybrids, at both N rates, though with an average difference of 4.4 Mg ha^{-1} in favor of the 390 kg N ha^{-1} rate (Table 3.14). Also, significant differences in stem and leaf biomass due to hybrids were observed at R3 and R5 at Zero N, but when the High N rate was applied, hybrid differences were only observed at R6. The High N rate increased stem and leaf biomass at V14 for DKC62-78, DKC67-72 and P0339 and at R3 and R5 for all hybrids. At the R5 stage, the High N rate increased stem and leaf biomass by an average of 3.0 Mg ha^{-1} (Table 3.14). At V7, DKC62-78 was the only hybrid that increased stem and leaf dry matter with the High N rate, suggesting a higher crop growth rate for this hybrid.

Grain biomass at R6, for the two hybrids common to all three environments, revealed no interactions between environment and any of the main effects (Table 3.7). Both the High N and High SR treatments increased shelled grain biomass (Table 3.15). When analyzing the six hybrids across both 2016 environments, both LEX2016 and HAR 2016 exhibited a significant N rate by seeding rate interaction ($p < 0.0001$). Increasing seeding rates at the High N rate treatment improved grain dry matter at both locations by an average 3.6 Mg ha^{-1} (Table 3.16). With Zero N, grain biomass was not affected by seeding rate.

Cob biomass was increased by the High N rate and the High SR for both 2016 environments, with maximum cob biomass reaching 1.3 Mg ha^{-1} at the highest plant densities (Table 3.17).

The High N rate increased husk biomass by 100 % (Table 3.18). Husk biomass differed for each hybrid, with A6517 producing the most (1.2 Mg ha^{-1}) and P2089 producing the least (0.5 Mg ha^{-1}).

3.2.5. Grain Harvest Index

Grain harvest index was calculated and statistically analyzed comparing LEX2015 and LEX2016 (Table 3.10). The environment effect on this parameter was significant and LEX2015 and LEX2016 were statistically analyzed separately. In 2015, a significant N rate by hybrid interaction ($p = 0.0519$) resulted in higher harvest index with the High N rate on both hybrids. However, no significant differences were observed between the High and Zero N rates for A6499 (Figure 3.3A).

In LEX2016, the significant N rate by hybrid interaction (p = 0.0011) resulted in significantly higher harvest index values when the High N rate was applied to all the hybrids under study (Figure 3.3B). In this regard, hybrid P0339AM exhibited the highest harvest index value of 0.70 (the range, due to hybrid, was from 0.62 to 0.70). Increasing the seeding rates in this environment significantly improved harvest index (0.67) at the High N rate (p = 0.0003). On the other hand, the High SR produced a significant decrease in harvest index at the Zero N rate (0.53; Figure 3.3C).

3.2.6. Stalk Weakness

Stalk weakness at LEX2016 (Figure 3.4) was significantly increased by the effect of actual plant population density at the High N rate treatment (p = 0.0004). In contrast, increasing measured plant populations did not significantly affect stalk weakness at the Zero N rate treatment (p = 0.1347). Overall, in all environments, combine harvest was conducted without lodging issues

Table 3.1. Nitrogen rate, seeding rate, hybrid, and soil type, taxonomic classification and properties for each environment.

Environment (latitude, longitude)	N Rate (kg N ha⁻¹)	Seeding Rate (seeds ha⁻¹)	Hybrids	Soil Type	Soil Taxonomy Classification	Properties
Lexington2015 (38˚12’96’’N, 84˚49’13’’W)	0 390	74000 148000	AgriGold A6499STXRIB AgriGold A6517VT3PRIB	Lowell-Bluegrass silt loam, 2-6 % slope	fine, mixed, active, mesic Typic Hapludalfs	well drained low runoff
Lexington2016 (38˚12’86’’N, 84˚49’12’’W)	0 390	74000 148000	AgriGold A6499STXRIB AgriGold A6517VT3PRIB DeKalb DKC62-78RIB DeKalb DKC67-72RIB Pioneer P0339AM Pioneer P2089AM	Lowell-Bluegrass silt loam, 2-6 % slope	fine, mixed, active, mesic Typic Hapludalfs	well drained low runoff
Hardin2016 (37˚61’67’’N, 85˚92’03’’W)	0 390	74000 148000	AgriGold A6499STXRIB AgriGold A6517VT3PRIB DeKalb DKC62-78RIB DeKalb DKC67-72RIB Pioneer P0339AM Pioneer P2089AM	Crider silt loam, 2-6 % slope	fine-silty, mixed, active, mesic Typic Paleudalfs	well drained low runoff

Table 3.2. ANOVA table summarizing sources of variation for each environment.

Environment	Source of variation	Grain Yield	Rows Ear^{-1}	Kernels Row^{-1}	Kernels Ear^{-1}	Kernels Ha^{-1}	Kernel Weight	Ear Length Ɨ	Tip Back Length Ɨ
	---p – value---								
Lexington 2015	ANOVA	<0.0001	0.1253	0.0703	0.1584	0.0069	0.2797	-	-
	N	0.0004	0.5000	0.1535	0.0276	0.0727	0.0789	-	-
	Hyb	0.9791	1.0000	0.8075	0.9113	0.8319	0.9208	-	-
	N*Hyb	0.3134	0.1056	0.0927	0.1738	0.8878	0.3910	-	-
	Pop	0.6646	0.2746	0.0082	0.0233	0.0013	0.5911	-	-
	N*Pop	0.3032	1.0000	0.1210	0.2826	0.0061	0.8412	-	-
	Hyb*Pop	0.8726	0.5614	0.9455	0.9691	0.3333	0.5700	-	-
	N*Hyb*Pop	0.0781	0.5614	0.6374	0.5461	0.0232	0.2770	-	-
Lexington 2016	ANOVA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	N	0.0001	0.0217	0.0177	0.0191	0.0033	0.0143	0.0161	0.0581
	Hyb	<0.0001	0.0001	0.2126	0.0152	0.2566	0.0384	0.2040	<0.0001
	N*Hyb	0.0260	0.7190	0.3220	0.4186	0.7793	0.0590	0.4036	0.0628
	Pop	0.1553	<0.0001	<0.0001	<0.0001	0.0615	<0.0001	<0.0001	0.0286
	N*Pop	<0.0001	0.0020	0.1609	0.1389	0.0023	<0.0001	0.6310	0.0364
	Hyb*Pop	0.0089	0.0232	0.1847	0.0624	0.5368	0.6668	0.3479	0.2897
	N*Hyb*Pop	0.0576	0.0135	0.7294	0.1782	0.4748	0.0753	0.7338	0.2671
Hardin 2016	ANOVA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
	N	0.0017	0.0444	0.0004	0.0003	0.0025	0.1609	0.0003	0.0955
	Hyb	0.0035	<0.0001	<0.0001	<0.0001	<0.0001	0.0098	<0.0001	<0.0001
	N*Hyb	0.5566	0.0420	0.2137	0.1045	0.0260	0.1812	0.4143	0.2436
	Pop	0.2233	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0161
	N*Pop	<0.0001	0.0080	0.0006	0.0028	<0.0001	0.0018	0.0020	0.0211
	Hyb*Pop	0.0027	0.0039	0.7445	0.1948	0.1900	0.0002	0.1036	0.0002
	N*Hyb*Pop	0.7220	0.0534	0.1938	0.5610	0.0588	0.1291	0.1505	0.0469

ƗLexington 2016 and Hardin 2016 data for this parameter.

Table 3.3. Seeding rate effect on Grain Yield, Kernels Ha^{-1} and 1000 Kernel Weight, within environment, hybrid and N rate.

Environment	Hybrid	N rate (kg/ha)	Seeding rate (seeds/ha)				p-value†	Seeding rate (seeds/ha)				p-value†	Seeding rate (seeds/ha)				p-value†
			74000		148000			74000		148000			74000		148000		
			Yield (Mg/Ha)					10^6 Kernels Ha^{-1}					1000 Kernel Wt. (g)				
Lexington 2015	A6499	0	10.3	b‡	8.9	B	0.0290	37.0	a	47.0	A	0.3440	247		264		
		390	16.9	a	18.5	A	0.0781	49.0	a	66.5	A	0.1602	326		304		
	A6517	0	9.4	b	10.4	B	0.4372	42.0	a	42.5	B	0.7952	255		208		
		390	17.3	a	17.2	A	0.9566	41.0	a	77.5	A	0.0261	338		344		
	Mean	0	9.8		9.6			39.5		44.8			251		236		0.6660
		390	17.1		17.9			45.0		72.0			332		324		0.7997
Lexington 2016	A6499	0	8.2	b	6.7	B	0.2166	41.0		45.0			263	b	263	A	1.0000
		390	15.2	a	19.1	A	0.0157	64.0		84.3			343	a	279	A	0.0073
	A6517	0	7.6	b	7.0	B	0.0809	59.0		63.3			270	b	246	A	0.0809
		390	17.7	a	20.6	A	0.0045	79.0		88.3			325	a	270	A	0.0488
	DKC62-78	0	8.1	b	6.8	B	0.0113	39.3		36.0			270	b	264	A	0.6401
		390	15.7	a	18.7	A	0.0104	69.3		80.6			381	a	284	A	0.0247
	DKC67-72	0	9.7	b	6.6	B	0.0205	50.0		39.6			255	b	270	A	0.5304
		390	17.5	a	19.8	A	0.0774	66.3		99.3			348	a	279	A	0.0644
	P0339	0	7.0	b	4.0	B	0.0320	42.3		30.3			256	b	247	B	0.5905
		390	14.5	a	18.6	A	0.0233	73.0		77.3			322	a	258	A	0.0759
	P2089	0	8.8	b	6.0	B	0.0313	41.6		35.3			233	b	253	A	0.3306
		390	19.3	a	19.1	A	0.8481	77.0		86.3			381	a	278	A	0.0360
	Mean	0	8.3		6.2			45.5	b	41.6	B	0.3388	258		257		
		390	16.7		19.3			71.4	a	86.0	A	0.0020	350		275		
Hardin 2016	A6499	0	8.5		6.0		0.8081	58.0	a	53.5	B	0.5045	291		276		0.0085
		390	11.7		13.8			60.2	a	71.7	A	0.2077	309		265		
	A6517	0	7.9		7.5		0.0403	43.5	a	55.7	B	0.1038	265		228		0.0025
		390	12.1		14.7			50.7	a	68.5	A	0.0350	298		267		
	DKC62-78	0	7.8		7.3		0.0239	37.5	b	48.2	B	0.1235	296		290		0.0496
		390	10.2		13.2			54.0	a	67.2	A	0.0207	317		291		
	DKC67-72	0	7.3		5.2		0.2958	45.0	a	47.5	B	0.5240	301		269		0.0039
		390	12.5		13.0			44.7	a	74.2	A	0.0126	353		250		
	P0339	0	7.2		3.5		0.3751	42.2	a	30.0	B	0.0641	286		284		0.3915
		390	10.3		12.5			50.6	a	75.6	A	0.0549	282		271		
	P2089	0	8.7		5.8		0.0513	47.2	a	52.0	B	0.6676	304		251		0.0003
		390	13.4		13.0			57.7	a	84.0	A	0.0307	322		239		
	Mean	0	7.9	b	5.9	B	<.0001	45.6		47.8			291	b	266	A	0.0001
		390	11.7	a	13.4	A	0.0002	53.0		73.5			314	a	264	A	<.0001

† Means in the same row with a p-value ≤ 0.10 are significantly different.
‡ Means are compared within each seeding rate.

Table 3.4. Predicted ear length response to actual plant population density for each hybrid.

					Target Population (plants ha^{-1})	
Environment	Hybrid	Equation	R^2	p-value†	74000	148000
					Predicted Ear Length (cm)	
Lexington 2016	A6499	$y = -0.00005481x + 21.84628$	0.25	0.0947	17.8	13.7
	A6517	$y = -0.00006550x + 24.31595$	0.38	0.0316	19.5	14.6
	DKC62-78	$y = -0.00003857x + 20.72138$	0.10	0.2978	17.9	15.0
	DKC67-72	$y = -0.00006244x + 23.40097$	0.41	0.0244	18.8	14.2
	P0339AM	$y = -0.00007215x + 23.29136$	0.40	0.0264	18.0	12.6
	P2089AM	$y = -0.00010475x + 27.94240$	0.62	0.0022	20.2	12.4
Hardin 2016	A6499	$y = -0.00006195x + 22.91810$	0.61	0.0003	18.3	13.7
	A6517	$y = -0.00005298x + 20.73572$	0.54	0.0011	16.8	12.9
	DKC62-78	$y = -0.00004788x + 20.96211$	0.39	0.0086	17.4	13.9
	DKC67-72	$y = -0.00005691x + 22.26689$	0.50	0.0020	18.1	13.8
	P0339AM	$y = -0.00007644x + 22.61475$	0.50	0.0041	17.0	11.3
	P2089AM	$y = -0.00007292x + 25.92270$	0.49	0.0025	20.5	15.1

† Significant values ($p \leq 0.10$) are highlighted.

Table 3.5. Predicted tip-back response to actual plant population density for each hybrid in Lexington and Hardin 2016.

Environment	Hybrid	Equation	R^2	p-value †	Target Population (plants ha^{-1})	
					74000	148000
					Predicted Tip Back (cm)	
Lexington 2016	A6499	$y = 0.00000426x + 0.43344$	0.10	0.3231	0.7	1.1
	A6517	$y = 0.00000551x + 0.16866$	0.12	0.2686	0.6	1.0
	DKC62-78	$y = -0.00000118x + 0.85889$	0.01	0.8021	0.8	0.7
	DKC67-72	$y = 0.00000533x -0.15070$	0.21	0.1389	0.2	0.6
	P0339AM	$y = -0.0000001155x + 1.43591$	0.00	0.9867	1.4	1.4
	P2089AM	$y = 0.00001081x + 1.16857$	0.15	0.2164	2.0	2.8
Hardin 2016	A6499	$y = 0.00000540x - 0.08140$	0.35	0.0155	0.3	0.7
	A6517	$y = -0.00000213x + 0.63883$	0.14	0.1462	0.5	0.3
	DKC62-78	$y = 0.00000210x + 0.12422$	0.09	0.2517	0.3	0.4
	DKC67-72	$y = 0.00000346x - 0.17364$	0.38	0.0107	0.1	0.3
	P0339AM	$y = -0.00000498x + 1.19791$	0.20	0.0999	0.8	0.5
	P2089AM	$y = 0.00000650x + 0.11772$	0.28	0.0333	0.6	1.1

† Significant values ($p \leq 0.10$) are highlighted.

Table 3.6. ANOVA table for stem and leaf biomass summarizing sources of variation for each environment.

Source of variation	stem & leaf biomass ha^{-1} p-value	Environment	Source of variation	stem & leaf biomass ha^{-1} p-value
env	<0.0001 Ɨ	Lexington 2015	n	0.0110 ‡
n	0.0003		hyb	0.5401
env*n	0.0323		n*hyb	0.6342
hyb	0.5893		pop	0.1490
env*hyb	0.3048		n*pop	0.1232
n*hyb	0.6844		hyb*pop	0.6134
env*n*hyb	0.5708		n*hyb*pop	0.7324
pop	0.0002		time	<0.0001
env*pop	0.1758		n*time	0.0028
n*pop	0.0195		hyb*time	0.3834
env*n*pop	0.4958		n*hyb*time	0.4352
hyb*pop	0.8237		pop*time	0.6533
env*hyb*pop	0.4697		n*pop*time	0.5050
n*hyb*pop	0.9918		hyb*pop*time	0.0090
env*n*hyb*pop	0.5312		n*hyb*pop*time	0.1692
time	<0.0001			
env*time	<0.0001	Lexington 2016	n	<0.0001 §
n*time	<0.0001		hyb	0.0819
env*n*time	<0.0001		n*hyb	0.6382
hyb*time	0.4575		pop	<0.0001
env*hyb*time	0.2498		n*pop	<0.0001
n*hyb*time	0.0984		hyb*pop	0.3683
env*n*hyb*time	0.9199		n*hyb*pop	0.3444
pop*time	0.0540		time	<0.0001
env*pop*time	0.4080		n*time	<0.0001
n*pop*time	0.1954		hyb*time	0.0002
env*n*pop*time	0.7444		n*hyb*time	0.0088
hyb*pop*time	0.0039		pop*time	<0.0001
env*hyb*pop*time	0.0718		n*pop*time	<0.0001
n*hyb*pop*time	0.1062		hyb*pop*time	0.9959
env*n*hyb*pop*time	0.5348		n*hyb*pop*time	0.9873

Ɨ ANOVA table for Lexington 2015 and Lexington 2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

‡ ANOVA table for Lexington 2015.
§ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

Table 3.7. ANOVA table for grain biomass summarizing sources of variation.

	2 hybrids, 3 environments	6 hybrids, 2 environments
Source of variation	Grain biomass ha^{-1} p-value	Grain biomass ha^{-1} p-value
env	0.5750 †	0.5558 ‡
n	0.0002	<0.0001
env*n	0.5053	0.0107
hyb	0.0002	0.1454
env*hyb	0.5053	0.0740
n*hyb	0.0002	0.9010
env*n*hyb	0.5053	0.2238
pop	0.0002	<0.0001
env*pop	0.3386	0.6599
n*pop	0.0055	<0.0001
env*n*pop	0.4920	0.7275
hyb*pop	0.2439	0.1602
env*hyb*pop	0.8532	0.9785
n*hyb*pop	0.9620	0.5955
env*n*hyb*pop	0.9933	0.8151

† ANOVA table for all environments. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.

‡ ANOVA table for Lexington and Hardin 2016. All 6 hybrids are included in the analysis.

Table 3.8. ANOVA table for cob biomass summarizing sources of variation.

Source of variation	Cob biomass ha^{-1} p-value	Environment	Source of variation	Cob biomass ha^{-1} p-value
env	0.5601 †	Lexington 2016	n	0.0057 ‡
n	<0.0001		hyb	0.3180
env*n	0.0075		n*hyb	0.3961
hyb	0.9790		pop	0.0330
env*hyb	0.1157		n*pop	0.4452
n*hyb	0.4729		hyb*pop	0.1427
env*n*hyb	0.7583		n*hyb*pop	0.2437
pop	0.0016			
env*pop	0.3685	Hardin 2016	n	0.006 §
n*pop	0.3640		hyb	0.4671
env*n*pop	0.9081		n*hyb	0.7258
hyb*pop	0.3346		pop	0.0126
env*hyb*pop	0.7041		n*pop	0.5198
n*hyb*pop	0.1399		hyb*pop	0.6350
env*n*hyb*pop	0.6299		n*hyb*pop	0.2874

†ANOVA table comparing Lexington and Hardin 2016. All 6 hybrids are included in the analysis.

‡ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

§ ANOVA table for Hardin 2016. All 6 hybrids are included in the analysis.

Table 3.9. ANOVA table for husk biomass summarizing sources of variation for the LEX2016 environment.

Source of variation	Husk biomass ha^{-1} p-value
n	0.0145 †
hyb	<0.0001
n*hyb	0.1655
pop	0.7231
n*pop	0.3913
hyb*pop	0.4478
n*hyb*pop	0.8492

†ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

Table 3.10. ANOVA table for grain harvest index summarizing sources of variation for each environment.

Source of variation	Grain harvest index p-value	Environment	Source of variation	Grain harvest index p-value
env	0.0070 †	Lexington 2015	n	0.1756 ‡
n	0.0018		hyb	0.1794
env*n	0.1437		n*hyb	0.0519
hyb	0.3579		pop	0.4717
env*hyb	0.0236		n*pop	0.1218
n*hyb	0.2895		hyb*pop	0.8815
env*n*hyb	0.0015		n*hyb*pop	0.9603
pop	0.9309			
env*pop	0.1787	Lexington 2016	n	0.0040 §
n*pop	0.0400		hyb	0.0299
env*n*pop	0.1687		n*hyb	0.0011
hyb*pop	0.3931		pop	0.4721
env*hyb*pop	0.6593		n*pop	0.0003
n*hyb*pop	0.9309		hyb*pop	0.3080
env*n*hyb*pop	0.8734		n*hyb*pop	0.3327

†ANOVA table comparing Lexington 2015 and Lexington 2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

‡ ANOVA table for Lexington 2015.

§ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

Table 3.11. Growth stage effect on stem and leaf dry matter per hectare, within N rate, for LEX2015.

LEX2015	Growth stage									
	V7		V14		R3		R5		R6	
N rate (kg N ha⁻¹)	Stem + Leaf Dry Matter (Mg ha⁻¹)									
0	1.9	C†	5.2	B	9.7	A	9.1	A	9.0	A
390	1.8	C	7.7	B	12.2	A	12.9	A	12.7	A
p-value	0.5113		0.0189		0.0115		0.0311		0.0176	

† Stem & leaf dry matter separated by N rate, for each growth stage. Means in the same row with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same column with a p-value ≤ 0.10 are significantly different.

Table 3.12. Seeding rate and growth stage effect on stem and leaf dry matter per hectare, within hybrid, for LEX2015.

LEX2015		Growth stage									
		V7		V14		R3		R5		R6	
Hybrid	Seed rate (seeds ha⁻¹)	Stem + Leaf Dry Matter (Mg ha⁻¹)									
A6499	74000	1.6	C†	7.4	B	10.7	A	10.6	A	10.4	A
	148000	2.2	C	5.4	B	12.5	A	11.6	A	11.4	A
	p-value	0.0132		0.0943		0.1686		0.6526		1.0000	
A6517	74000	1.5	C	5.2	B	9.9	A	11.0	A	10.3	A
	148000	2.0	C	7.7	B	10.6	A	10.9	A	11.1	A
	p-value ‡	0.1317		0.112		0.4102		0.9779		0.6622	

† Stem & leaf dry matter separated by hybrid, for each seeding rate and growth stage. Means in the same row within seeding rate with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same column within hybrid and growth stage with a p-value ≤ 0.10 are significantly different.

Table 3.13. Nitrogen rate, seeding rate and growth stage effects on stem and leaf dry matter per hectare, for LEX2016.

LEX 2016	Growth stage																			
	V7				V14				R3				R5				R6			
	Stem + Leaf Dry Matter (Mg ha^{-1})																			
SR / N rate	74000		148000		74000		148000		74000		148000		74000		148000		74000		148000	
0	0.83	B[†]	1.17	A	3.62	B	4.20	A	5.83	B	6.87	A	4.68	B	5.59	A	4.36	A	4.99	A
390	0.88	b	1.65	a	5.31	b	7.34	a	9.06	b	12.38	a	7.22	b	8.84	a	5.95	a	6.19	a
p-value ‡	0.5465		<.0001		<.0001		<.0001		<.0001		<.0001		<.0001		0.0016		0.0003		<.0001	

† Stem & leaf dry matter separated by N rate and growth stage for each seeding rate. The N rate by seeding rate by growth stage interaction was significant (p = 0.0013). Means are compared within each N rate and growth stage. Means in the same row with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same column within growth stage and seed rate with a p-value ≤ 0.10 are significantly different.

Table 3.14. Hybrid and growth stage effects on stem and leaf dry matter per hectare, within N rate, for LEX2016.

LEX2016		Hybrid												
		A6499		A6517		DKC62-78		DKC67-72		P0339		P2089		p-value [‡]
N rate (Kg N Ha^{-1})	Growth stage	Stem + Leaf Dry Matter (Mg ha^{-1})												
	V7	1.2	D[†]	1.0	D	0.9	D	1.0	C	0.9	D	1.0	C	ns[*]
	V14	4.4	C	4.4	C	3.4	C	4.5	B	3.0	C	3.8	B	ns
0	R3	7.4	A	6.9	A	5.6	A	6.7	A	5.3	A	6.2	A	0.0861
	R5	5.9	B	5.2	BC	4.5	B	5.2	B	4.3	B	5.7	A	0.0107
	R6	5.0	C	6.1	AB	3.8	BC	4.6	B	4.1	B	4.4	B	ns
	V7	1.3	D	1.0	D	1.2	D	1.4	D	1.5	D	1.2	C	ns
	V14	6.3	C	5.5	C	6.7	BC	6.5	C	6.5	B	6.5	B	ns
390	R3	11.1	A	11.8	A	10.8	A	10.2	A	9.1	A	11.4	A	ns
	R5	8.5	B	8.9	B	7.4	B	8.6	B	7.0	B	7.9	B	ns
	R6	5.9	C	6.4	C	5.8	C	7.2	C	4.2	C	6.9	B	0.0038
		p-value [§]												
	V7	ns		ns		0.0703		ns		ns		ns		
0	V14	ns		ns		0.0148		0.0100		<.0001		ns		
vs.	R3	0.0216		<.0001		0.0023		0.0163		<.0001		0.0647		
390	R5	0.0046		0.0003		0.0035		0.0010		0.0355		0.0627		
	R6	ns		ns		ns		0.0041		ns		0.0588		

[†] *Stem & leaf dry matter separated by N rate for each growth stage and hybrid. For both N treatments, the growth stage by hybrid interaction was significant, therefore, means are compared within each hybrid. Means in the same column with different letters are significantly different ($p \leq 0.10$).*

[‡] *Means in the same row within N rate and growth stage with a p-value ≤ 0.10 are significantly different.*

[*] *ns = not significant at the $\alpha = 0.10$ significance level.*

[§] *Means comparison among N rate treatments within hybrid for each growth stage with a p-value ≤ 0.10 are significantly different.*

Table 3.15. Nitrogen and seeding rate effects on grain dry matter per hectare across two hybrids and three environments.

All Environments	Seeding rate (seeds ha^{-1})			
	74000		148000	
N rate (kg N ha^{-1})	Grain Dry Matter (Mg ha^{-1})			
0	9.2	ns*	9.9	
390	14.1	B†	18.0	A

†N rate by seeding rate interaction for grain dry matter was significant. Means in the same row within N rate with different letters are significantly different ($p \leq 0.10$).

** ns = not significant ($p \leq 0.10$).*

Note: Hybrids A6499 and A6517 were used in this analysis since planted in 2015 and 2016.

Table 3.16. N and seeding rate effects on grain dry matter per hectare for LEX2016 and HAR2016.

LEX2016-HAR2016	Seeding rate (seeds ha^{-1})			
	74000		148000	
N rate (kg N ha^{-1})	Grain Dry Matter (Mg ha^{-1})			
0	8.3	ns*	8.5	
390	13.7	B†	17.3	A

† For LEX2016 and HAR2016, N rate by seeding rate interaction for grain dry matter was significant. Means in the same row within N rate with different letters are significantly different ($p \leq 0.10$).

** ns = not significant ($p \leq 0.10$).*

Note: All six hybrids used in the analysis.

Table 3.17. N and seeding rate effects on cob dry matter per hectare across hybrids for LEX2016 and HAR2016.

	N rate (kg N ha^{-1})				Seed rate (seeds ha^{-1})			
Environment	0		390		74000		148000	
	Cob Dry Matter (Mg ha^{-1})							
LEX2016	0.9	B †	1.6	A	1.2	B †	1.3	A
HAR2016	1.0	B	1.4	A	1.1	B	1.3	A

††N rate and seeding rate effect for cob dry matter was significant. Means in the same row within environment with different letters are significantly different ($p \leq 0.10$).

Table 3.18. N rate and hybrid effects on husk dry matter per hectare across seeding rates, for LEX2016.

LEX2016	Hybrid													
	A6499		A6517		DKC62-78		DKC67-72		P0339		P2089		Mean	
NR (kg N ha^{-1})														
	Husk Dry Matter (Mg ha^{-1})													
0	0.5		0.8		0.5		0.6		0.4		0.4		0.5	B†
390	1.0		1.2		1.1		1.1		0.8		0.6		1.0	A
Mean	0.8	B‡	1.0	A	0.8	B	0.9	B	0.6	C	0.5	D		

† For LEX2016, N rate effect for husk dry matter was significant. Means in the same column with different letters are significantly different ($p \leq 0.10$).

‡ Hybrid effect for husk dry matter was significant. Means in the same row with different letters are significantly different ($p \leq 0.10$).

Figure 3.1: Monthly average temperature (A), precipitation (B) for Lexington 2015, Lexington 2016 and the past 30 years. Monthly average temperature (C), precipitation (D) for Hardin 2016 and the past 30 years.

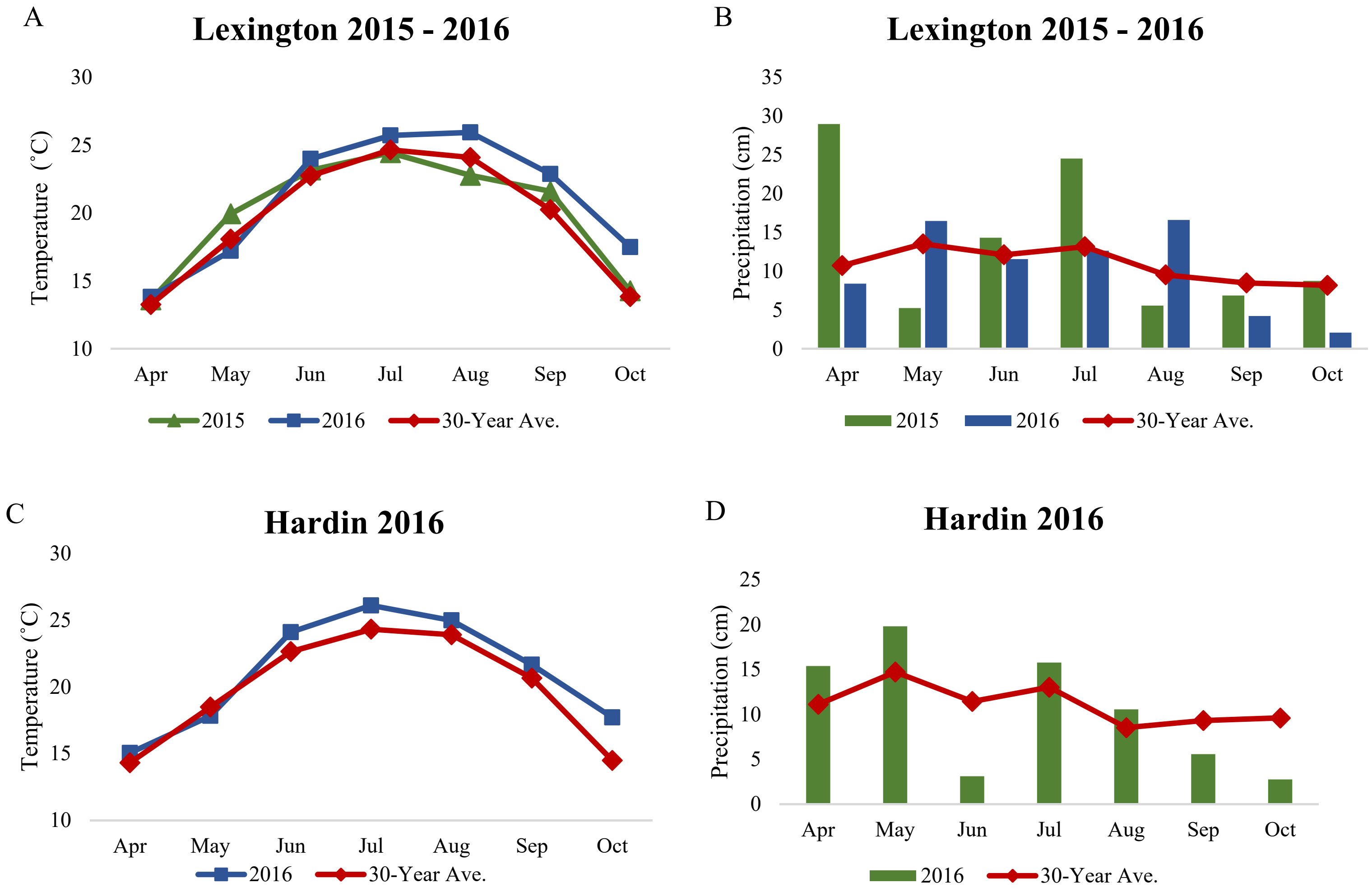
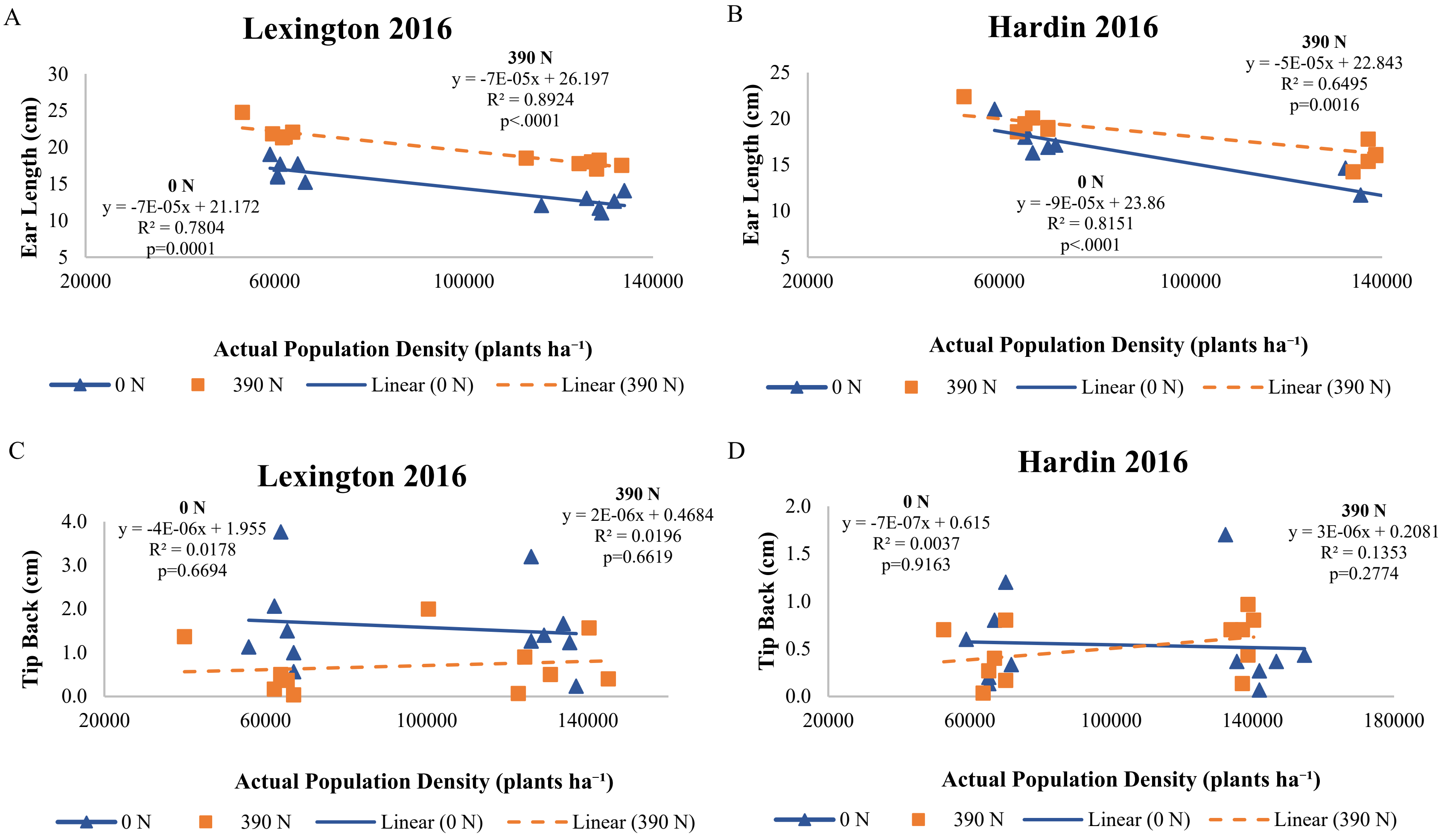


Figure 3.2. Mean ear length (cm) at 0 or 390 kg N ha^{-1} as a function of actual plant population density for Lexington 2016 (A) and Hardin 2016 (B). Mean tip back length (cm) at 0 or 390 kg N ha^{-1} as a function of actual plant population density for Lexington 2016 (C) and Hardin 2016 (D). Means are for each hybrid (A6499, A6517, DKC62-78RIB, DKC67-72RIB, P0339AM and P2089AM).



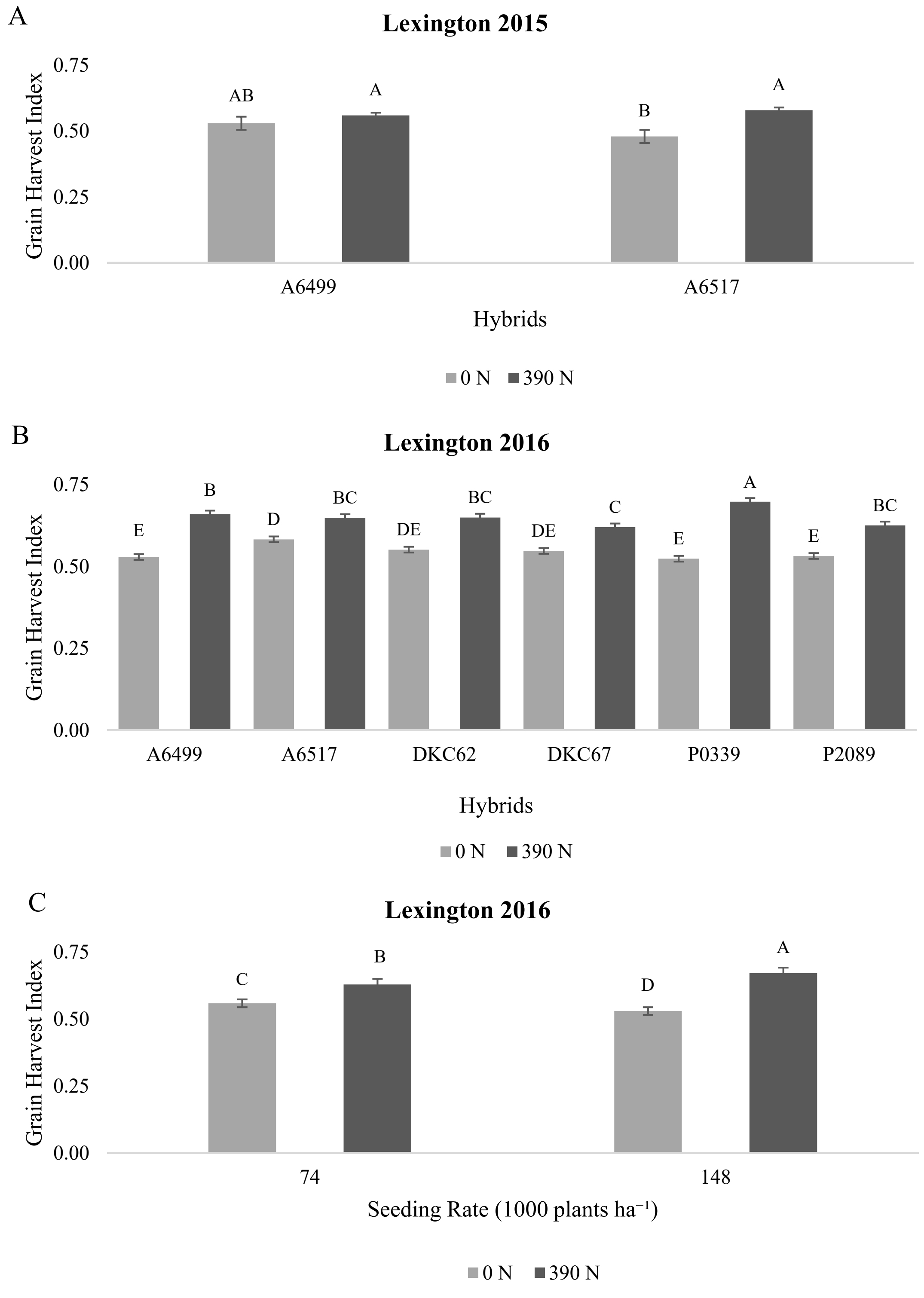


Figure 3.3. Grain harvest index at Lexington 2015 (A) was significantly affected by a N rate by hybrid interaction ($p = 0.0519$). Grain harvest index at Lexington 2016 (B) was significantly affected by a N rate by hybrid interaction ($p = 0.0011$) and by a N rate by seeding rate interaction (C; $p = 0.0003$). Means overtopped by different letters are significantly different ($p \leq 0.10$).

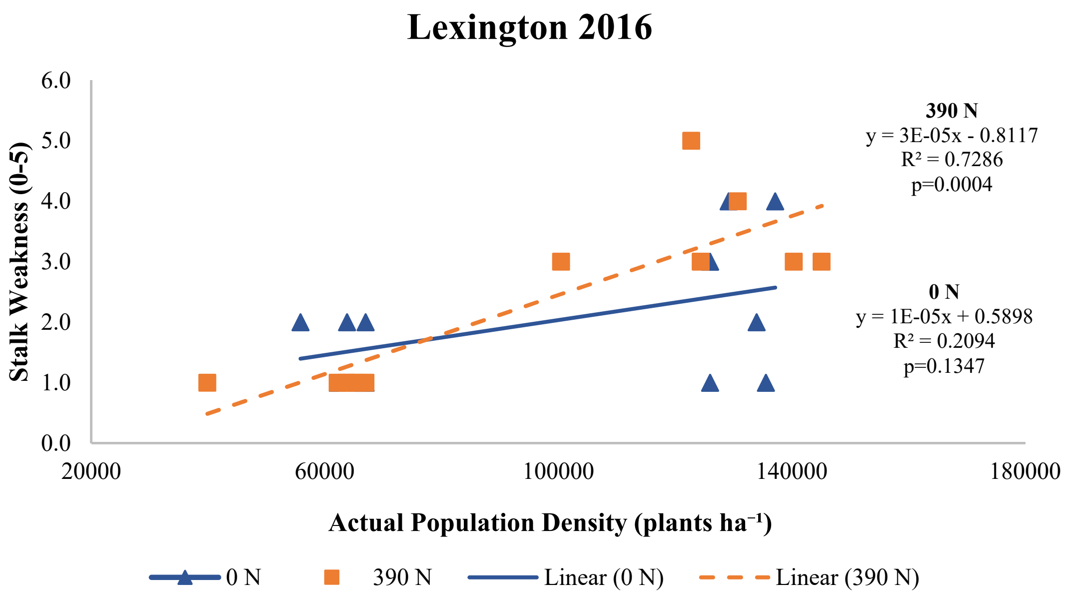


Figure 3.4. Stalk weakness rating at 0 or 390 kg N ha^{-1} as a function of actual plant population density for Lexington 2016. Means are for each hybrid (A6499, A6517, DKC62-78RIB, DKC67-72RIB, P0339AM and P2089AM). Stalk weakness was rated on a 0-5 scale, with 0 representing unbreakable stalks when applying pressure up to a 30˚ angle, and 5 representing breakable stalks with that same pressure.

3.3. Discussion

3.3.1. Grain Yield Responsiveness

The 390 kg N ha^{-1} treatment increased grain yield within each environment, for both seeding rates and all hybrids tested. In LEX2015, grain yield from the High N treatment was 81% above the Zero N treatment when averaged across seeding rates and hybrids (relative increase was calculated as the difference between the mean of all treatments with the High N rate and the mean with the Zero N rate treatments and expressed as a percentage of the lowest N rate (Crozier et al., 2014)). At LEX2016 and HAR2016, grain yield from the High N treatment was 149% and 82% above the Zero N treatment, respectively. These general results exceeded and are consistent with the findings of Crozier et al., (2014) in North Carolina and Ciampitti et al. (2011) in Indiana.

In addition to these results, Massignam et al., (2009) observed maize grain yield to be highly responsive to N supply, obtaining an average of 2.1 to 12.5 Mg ha^{-1} with N rate treatments ranging from 0 to 300 kg N ha^{-1} and a seeding rate of approximately 70,000 plants ha^{-1}. The authors attributed differences in grain yield response to differences in dry matter production among treatments. In view of these findings, higher seeding rates would therefore be expected to improve maize biomass production and thus grain yield, provided N conditions were non-limiting. Results from this research support this hypothesis. Grain yield at 148,000 plants ha^{-1} averaged 17.9, 19.3 and 13.4 Mg ha^{-1} at LEX2015, LEX2016 and HAR2016, respectively, when supplied with 390 kg N ha^{-1}, greatly exceeding grain yield values reported by Massignam et al. (2009).

In effect, both N rate and seeding rate strongly impacted maize grain yield in all Kentucky environments studied. Increasing plant population improved grain yield by 1.6 Mg ha^{-1} at the High N rate, with hybrid A6499, at LEX2015. The High SR, at the High N rate, in LEX2016 increased maize yield by an average of 3.2 Mg ha^{-1} (ranging from 2.3 to 4.1 Mg ha^{-1}) for five of the six hybrids tested. Maximum grain yield production (20.6 Mg ha^{-1}) was obtained in this environment when combining the highest N and seeding rate.

At HAR2016, the High SR and N rate combination increased yields by 2.5 Mg ha^{-1} for four of six hybrids in this lower-yielding (ranging from 12.5 to 14.7 Mg ha^{-1}) environment. The lower yields at HAR2016 can be explained in part by some external abiotic/biotic factors that occurred during the growing season. The corn suffered early water deficit prior to V9 (Abendroth et al., 2011), with leaf rolling occurring in most plots and being more severe in the High SR, High N rate plots. According to Duvick (2005), newer commercial hybrids expressed greater leaf rolling when compared under different drought stress management scenarios at several growth stages. Barker et al., (2005) also suggested that elite corn hybrids can utilize leaf rolling to reduce light interception and water use. During vegetative growth stages, maize plants are somewhat tolerant of stresses. However, cell expansion depends on how turgid the plant is and therefore some of the effects that might develop as a result could be reductions in leaf area and plant height (Barker et al.,2005) by R1. As a consequence, reductions in plant biomass can take place because less solar radiation can be intercepted. Also important to consider is the fact that the soil at HAR2016 is a Crider silt loam, with moderate (about 7.6 inches) available water storage in the profile (Web

Soil Survey, 2016). Lastly, another factor that could have contributed to the general lower yield values observed for HAR2016 is the late disease pressure that the plots experienced around R5 (kernel dent, Abendroth et al., 2011). After the dry weather, abundant rainfall later in July generated favorable conditions for some corn foliar diseases. At HAR2016 gray leaf spot (*Cercospora zeae-maydis*) was mostly observed and, in hindsight, a foliar fungicide should have been applied. Gray leaf spot is one of the most common foliar diseases of corn each season (Bradley, 2016).

Ciampitti et al., (2013) found an improvement in grain yield response to nitrogen (224 vs. 0 kg N ha^{-1}) at 79,000 and 104,000 seeds ha^{-1}, compared with a lower seeding rate (54,000 seeds ha^{-1}). However, maximum grain yield of 13 Mg ha^{-1} occurred at the intermediate seeding rate of 79,000 seeds ha^{-1}. A recent meta-data analysis by Assefa et al., (2016), including several U.S. states, concluded that grain yield showed a persistent upward trend (>13 Mg ha^{-1}) to increasing plant densities (up to 123,500 plants ha^{-1}). Woli et al., (2016) observed that the grain yield response to N supply for hybrids circa 2000 exhibited a steady increase with no signs of a plateau (with 228 kg N ha^{-1} and 84,000 plants ha^{-1}).

These results, along with the findings from other current research, suggest opportunities exist for further grain yield improvement through increased plant densities, provided high yielding environments and hybrids are used.

3.3.2. Grain Yield Components

The High SR generally decreased kernels ear^{-1} compared with the Low SR. Reduction in kernels ear^{-1} was less dramatic at the High N rate than at the Zero N rate.

In general, the High N rate increased kernels ear^{-1} in all environments when analyzed across seeding rates and hybrids. At LEX2015, the High N rate increased kernels ear^{-1} by 32%, on average, when compared to the Zero N treatment. In 2016, kernels ear^{-1} increased an average of 62 and 34% at LEX2016 and HAR2016, respectively, when supplied with the High N rate. More interestingly, in the 2016 environments the combined effect of N and seeding rates strongly impacted the number of kernels ear^{-1}. The High SR and Zero N rate decreased kernels ear^{-1} by 83 and 69% at LEX2016 and HAR2016, respectively, compared with the Low SR at Zero N. In contrast, at the High N rate, the High SR reduced kernel number ear^{-1} by 35 and 28% at LEX2016 and HAR2016, respectively. Clearly, the resulting N deficiency delivered by the Zero N treatment together with increased plant competition in the higher density treatments (Andrade et al., 2002) severely reduced the number of kernels ear^{-1}. Particularly stressful conditions during the silking period can greatly reduce kernel number per ear (Abendroth et al., 2011).

As described by Amanullah et al. (2016), the increased kernel number ear^{-1} when higher N rates are applied to maize might be partially explained by an extended growing period and thus more photosynthate is available to be partitioned to the kernel during reproductive growth. Since assimilates from photosynthesis produced during maize vegetative stages are essential for kernel development, a strong association between kernel number per plant (uppermost ear) and plant growth rate during the critical period for kernel set was proposed by several researchers (Echarte et al., 2013; Vega et al., 2001; Andrade et al., 1999; Tollenaar et al., 1992). In this regard, plant population density directly impacts the amount of dry matter per plant that can potentially be partitioned to reproductive structures (Andrade et al., 1999). Ultra-high plant densities decrease plant growth rate as resources per plant decline and, therefore, less assimilate is partitioned to the developing ear. In the present study, the High N treatment apparently mitigated, to some extent, the reduction in kernel number ear^{-1} produced by supraoptimal plant densities. However, intra-plant competition still reduced growth rate per plant and consequently limited dry matter distribution to the ear.

Kernels per ear is a function of rows ear^{-1} and kernels row^{-1}. Kernels row^{-1} was affected by N rate and seeding rate more than rows ear^{-1}.

Rows per ear significantly declined only at the Zero N rate and High SR, in most comparisons about 3 rows ear^{-1}, on average. However, the high N rate kept the number of rows ear^{-1} from decreasing at supraoptimal plant densities. Rows per ear increased with N rate and was maximized at 134 kg N ha^{-1} in North Carolina (Crozier et al., 2014).

The High N rate increased kernels row^{-1} by 24, 44 and 26% at LEX2015, LEX2016 and HAR2016, respectively. On the other hand, High SR decreased kernels row^{-1} by 32 and 17% at the Zero and High N rates, respectively, at HAR2016. High SR also reduced kernels row^{-1} at LEX2016. Kernels row^{-1} decreased when plant densities increased from 55,000 to 95,000 plants ha^{-1} in Argentina and France (Otegui and Bonhomme, 1998).

The smaller reduction in the number of kernels per row at High SR with the higher N rate can be partly explained by an increment in the number of potential kernels per ear (Echarte et al., 2013). However, doubling the seeding rate to 148,000 seeds ha^{-1}

increased plant competition to a point where greater N availability was not sufficient to guarantee assimilate distribution to all potential kernels in each ear. These outcomes were also observed as a function of higher actual plant populations, as ear length was reduced at both Zero and High N rates. The High N rate, however, did increase ear length.

In sum, the number of kernels per row was the component that most influenced kernel number per ear. Nitrogen deficiency possibly reduced kernels row^{-1} by negatively impacting potential kernel number per row. The High N rate was intended to be non-limiting to yield and did increase kernels row^{-1}. However, the High SR still reduced kernels row^{-1}, implying that either N or another factor was limiting potential kernel number. Our hypothesis was that the High N rate would increase kernels ear^{-1} at High SR and these observations did not support our original hypothesis.

While kernels ear^{-1} decreased at High SR, kernels ha^{-1} increased with the High SR and High N rate treatment combination. However, at Zero N rate, kernels ha^{-1} was not significantly different at either SR. Effectively, the proposed hypothesis was confirmed, as supraoptimal plant densities increased kernel number per unit area when applying non-limiting N.

When comparing seeding rate effects, kernel mass declined by an average of 21% at LEX2016 with the High SR and High N rate treatment combination. However, kernel mass was not significantly different by seeding rate at the Zero N rate. In HAR2016, kernel mass declined 9 and 16% with the High SR at Zero and High N rates, respectively. When comparing N rate effects, High N increased kernel mass at the Low

SR in most comparisons. However, at the High SR, the High N rate increased kernel mass only for hybrid P0339.

These findings generally confirmed our hypothesis that the higher N rate would increase kernel weight and a minor decline would occur as seeding rates doubled.

A reasonable argument to explain the decline in kernel mass with the High SR and High N rate treatment combination would be based on the observation that a greater number of kernels per unit area was observed with this treatment combination. Thus, the decrease in kernel weight was a consequence of a compensatory mechanism to a higher kernel number under the High SR. Evidently, the increased grain yield at the higher SR and N rate was mainly the result of increased kernel number per hectare. Final kernel mass adjusted in order to make up for kernel number improvement.

Maize kernel size is influenced by growth rate and filling period duration. When maize is planted at higher densities, kernel mass can be decreased (Lemcoff and Loomis, 1986; Poneleit and Egli, 1979).

Kernel growth rate is strongly related to both the number of endospermic cells and the amount of starch granules that are formed during the *lag* phase (Capitanio et al., 1983). As a result, the sink capacity (or strength) of each grain is determined at this point. These formative structures influence photosynthate partitioning to the developing kernel. Kernel growth rate explained most of the differences found in kernel weight among hybrids released in different decades (Echarte et al., 2013), suggesting that this component is related to genetic information and is highly associated to the potential kernel size established during the *lag* phase.

If positive increments in maize grain yield continue to be highly associated with increasing plant densities in the next decades, improvement in kernel size via breeding techniques that focus mainly on kernel sink capacity could be a decisive factor in maintaining those positive yield increments.

3.3.3. Biomass

Stem and leaf biomass was influenced by environment when comparing LEX2015 and LEX2016. At both these environments, treatment effects on stem and leaf dry matter interacted with growth stage. The High N rate increased stem and leaf biomass at all growth stages studied, except V7 in 2015. This observation would suggest that at early stages soil N was sufficient to sustain biomass accumulation in the Zero N treatment. The highest dry matter difference was observed at V14, with the High N rate giving a 48% increase in stem and leaf biomass above the Zero N rate. Stem and leaf biomass was maximized at R3 in both N treatments. Furthermore, R6 fodder biomass was found to be 41% greater with High N. These findings agree with those of Ciampitti and Vyn (2011), who observed increased stover biomass during reproductive stages in response to applied N. Hybrid marginally influenced stem and leaf biomass from V7 to V14, and interacted with seeding rate. Increased seeding rates increased dry stem and leaf biomass at these two growth stages for hybrid A6499.

At LEX2016, stem and leaf biomass increased with increased plant density at growth stages V7, V14, R3 and R5 at both N rates. At physiological maturity, no variation in biomass due to hybrid was observed at the higher plant density, within an N rate. These results would indicate significant remobilization to reproductive structures that minimized the influence of increased plant density on early dry stem and leaf biomass by the end of the growing season. On the other hand, the High N rate increased stem and leaf biomass accumulation at every growth stage and seeding rate, and the only exception was observed at V7 with Low SR. Once more, available soil N was apparently sufficient to achieve similar plant growth at early developmental stages when seeded at the lower seeding rate. Similar results were obtained by Barbieri et al. (2013) in Argentina, where they observed an increase in aboveground dry matter accumulation at most post-anthesis phenological stages in response to N application and no variation in biomass at early vegetative stages.

Dry stem and leaf biomass was maximized at R3 for both N and both seeding rates, declining at R5 and R6. This decline in dry matter can be primarily due to reallocation of N and other nutrients from stem and leaves to reproductive structures as kernel development becomes the priority (Abendroth et al., 2011). Also, at these late growth stages leaf senescence progresses and the process can be accelerated by higher plant densities (Abendroth et al., 2011; Borrás et al., 2003).

Stem and leaf biomass was also maximized at R3 for each hybrid, at both Zero and High N rates. All hybrids exhibited a response to applied N at growth stages R3 and R5. The High N treatment increased dry stem and leaf biomass by an average of 70% above the Zero N rate at R3 and by 58% at the R5 growth stage. By physiological maturity, the majority of the hybrids exhibited no differences in stem and leaf dry matter due to the N treatments.

Overall, stem and leaf biomass increased with the High N rate and the High SR. Increases in both these treatment factors caused a greater canopy growth and thus increased plant biomass accumulation (Ciampitti et al., 2013; Yan et al., 2016).

Grain biomass increased in the High N rate and High SR treatment combination, in all environments. The High SR increased dry grain biomass by 24 and 27% at LEX2016 and HAR2016, respectively, at the High N rate. Seeding rate did not affect grain biomass at the Zero N rate. When comparing N rates, the High N rate increased grain biomass by 132 and 81% in LEX2016 and HAR2016, respectively, at the High SR. As observed by Ciampitti et al. (2013), grain biomass responded in greater measure to N supply when compared to stem and leaf biomass.

Cob and husk dry matter at maturity increased with the High N treatment. In LEX2016 and HAR2016, dry cob biomass ha^{-1} increased 78 and 40%, respectively. Husk biomass doubled when the High N rate was applied. On the other hand, increased seeding rate increased cob biomass by 8 and 18% in the two environments, respectively, regardless the N treatment. However, grain biomass was more responsive than these two fractions to supplied N.

3.3.4. Grain Harvest Index

The harvest index of modern hybrids has increased over the decades (Sinclair, 1998). In addition, N supply is strongly associated with maize grain yield and thus grain harvest index. Non-limiting N increased grain harvest index in all hybrids tested at LEX2015 and LEX2016. Furthermore, the harvest index response to N was higher with increased plant density in 2016. A maximum grain HI of 0.67 was observed for the

High N and High SR treatment combination. Apparently, the higher stem and leaf biomass obtained during the vegetative growth and up to R5 with increased plant density and N rate contributed to kernel biomass accumulation by physiological maturity. Also, modern hybrids exhibit longer seed filling periods (Echarte et al., 2013; Duvick, 2005). A longer seed filling period with the non-limiting N treatment could have positively impacted grain dry matter.

On the contrary, the High SR and Zero N treatment combination exhibited the lowest grain harvest index (0.53). A decrease of 26% in grain harvest index was observed in LEX2016 when comparing High SR and High N with High SR and Zero N. Several studies reported comparable results (Ciampitti et al., 2013; Ciampitti and Vyn, 2011; Sangoi, 2000; Massignam et al., 2009).

3.3.5. Stalk Weakness

Modern hybrids show more resistance to stalk lodging. The breeding of more stress tolerant maize hybrids has allowed seeding rates to increase in the last decade so as to maximize grain yield (Reeves and Cox, 2013; Duvick, 2005).

At LEX2016, stalk weakness at maturity increased with increased SR, regardless of hybrid, when the High N rate was applied. However, weaker stalks as a result of increased plant population did not impose combine harvest limitations at any of the environments studied. As observed by Stanger and Lauer (2006), newer hybrids (Bt hybrids) require higher plant densities to maximize grain yield since they exhibit reduced stalk lodging.

3.4. Summary

High input production systems have accompanied increases in grain yield in past decades and will still be vital if maximization of crop yields remains the main objective in the future. Maize grain yield is not the exception.

Grain yield increases in maize in response to higher plant densities has been the focus of several research studies in recent years (Tokatlidis and Koutroubas, 2004; (Maddonni et al. 2006; Boomsma et al. 2009; Raymond et al. 2009; Barbieri et al. 2008; Ciampitti et al. 2013; Lamm and Trooien, 2001). However, few of these have investigated the combined effect of seeding rates up to 148,000 ha^{-1} and non-limiting N on potential yield.

Results from this research corroborate our main hypothesis. Intensified inputs have increased maize grain yield, essentially through increased number of kernels per ha^{-1}. However, kernels per ear and kernel size restricted further yield increases.

Maize plant densities will follow the current upward trend if grain yield components show no variation (Egli, 2015; Assefa et al., 2016). Future breeding developments should focus on kernel mass and kernels per ear.

Chapter 4

4. Nutrient Uptake and Accumulation

4.1. Objectives

Increased biomass accumulation and grain yield in modern maize hybrids may drive more nutrient uptake and removal. The primary objective was to investigate how Low and high plant densities, combined with zero and non-limiting N rates, affect nutrient accumulation throughout the growing season. In addition, we want to understand if these treatment combinations also produce differences in kernel nutrient removal. Specific hypotheses were:

a) Stem and leaf N uptake will increase when combining the High N rate and High SR.
b) Grain N removal per hectare will increase at High SR and High N rate. Phosphorus and potassium removal will be increased with increments in N removal. Grain N accumulation per plant is expected to decrease with High SR. However, this parameter will increase with the application of the non-limiting N rate.
c) Cob and husk nutrient accumulation will mirror grain N accumulation.
d) High SR combined with the limiting N rate, will cause nitrogen deficiency to occur higher on the corn plant, closer to the ear leaf.

4.2. Results

4.2.1. Stem and Leaf Nitrogen Accumulation

Total inorganic soil N (NH_4-N and NO_3-N) was below 10 ppm at LEX2015 and was lower than 48 ppm at LEX2016 and HAR2016 (Table 4.1). Overall, at all environments, the first 15 cm of soil contained the highest quantity of available N.

Stem and leaf N uptake per hectare was significantly affected by environment ($p < 0.0001$). Therefore, LEX2015 and LEX2016 were analyzed separately (Table 4.2).

At LEX2015, a significant three-way interaction of hybrid, seeding rate and growth stage occurred ($p = 0.0329$) for stem and leaf N. Hybrid interactions were of magnitude, not of response; therefore, the two-way interactions of N rate and seeding rate ($p = 0.0362$) and N rate by growth stage ($p < 0.0001$) were evaluated.

At LEX2016, stem and leaf N uptake was significantly affected by the three-way N rate by seeding rate by growth stage ($p < 0.0001$) and hybrid by N rate by growth stage ($p = 0.0089$) interactions. Once again, hybrid interactions were of magnitude, not of response. Focus was placed on the interactions between N rate, seeding rate and growth stage on stem and leaf N accumulation.

As expected, N application significantly increased stem and leaf N uptake at all growth stages in both LEX2015 and LEX2016 trials. On average, across seeding rates, the High N treatment increased stem and leaf N accumulation by 52, 139, 146, 190 and 167 % at V7, V14, R3, R5 and R6, respectively when compared to the Zero N rate in the LEX2015 trial (Table 4.13).

In the LEX2016 trial, the High N rate caused greater accumulation of N in stem and leaf biomass at both seeding rates when compared to the Zero N treatment (Table 4.14). On average, N stem and leaf uptake was 111, 261, 405, 312 and 157 % higher at

V7, V14, R3, R5 and R6, respectively, with Low SR. On the other hand, High SR and High N increased stem and leaf N uptake by 274, 450, 603, 473 and 107 % at V7, V14, R3, R5 and R6, respectively. The magnitude of the increments in stem and leaf N uptake above the Zero N treatment were greater for the High SR when compared to the Low SR. The trend was consistent until physiological maturity.

When compared with Low SR, higher plant population densities increased N accumulation in fodder biomass by 92% at V7, 42% at V14 and 27% at R3 when supplied with 390 kg N ha^{-1} in the LEX2016 trial (Table 4.14). At the R5 growth stage, however, no significant differences in stem and leaf N uptake were observed due to seeding rate. Moreover, at R6, a 20% decrease in accumulated stem and leaf biomass N was detected when comparing High to Low SR.

Maize stem and leaf N uptake exhibited maximum values at the V14 and R3 growth stages (the period bracketing silking), followed by a gradual decline at later growth stages. Overall, this trend was observed in both environments, for both levels of N and both seeding rates. Also, most hybrids presented the same N accumulation pattern (Table 4.13, 4.14).

4.2.2. Husk and Cob Nitrogen Uptake

Husk N uptake was analyzed at R5 in the LEX2015 trial and at R3, R5 and R6 in the LEX2016 trial (Table 4.3).

In the LEX2015 trial, husk N accumulation was significantly affected by a three-way N rate, hybrid and seeding rate interaction ($p = 0.0255$). The High N treatment increased husk N uptake by 140% at Low SR of A6499. However, no significant differences in accumulated husk N were observed among seeding rates for A6517 (Table 4.15).

For LEX2016, several interactions between N rate and seeding rate on stem and leaf N accumulation occurred. We focused on the significant two-way seeding rate by growth stage ($p = 0.0213$) interaction (Table 4.3). In this interaction, High SR decreased N accumulation in husk biomass at R3 by 15% compared with Low SR. As the growing season progressed, differences among seeding rates disappeared and no significant seeding rate effect on husk N uptake was observed at R5 and R6.

Cob N accumulation was evaluated at R5 in LEX2015 and at R5 and R6 in LEX2016 (Table 4.4). Main treatment factors and their interactions on cob N accumulation were not significant in LEX2015.

In the LEX2016 experiment, however, numerous interactions concerning all treatment factors affected cob N accumulation. Nonetheless, given its importance, the two-way seeding rate by growth stage interaction ($p = 0.0813$) was further explored. In this environment, as seeding rate increased, a 24% rise was observed in cob N uptake at R5. Similarly, at physiological maturity, High SR increased cob N accumulation by 35% when compared to Low SR (Table 4.16).

4.2.3. Cob + Grain N Uptake at R3

At R3, the cob fraction and developing grain component were analyzed together for N uptake in both Lexington environments. Since environment significantly interacted with N rate, hybrid and seeding rate ($p = 0.0936$), N and seeding rate effects on cob plus grain N uptake at R3 were analyzed by environment (Table 4.5).

A three-way N rate, hybrid and seeding rate interaction ($p = 0.0293$) significantly affected N accumulation in the cob plus grain component at R3 in LEX2015.

The High N increased N accumulation in cob plus grain at both seeding rates when compared with Zero N. High N increased cob plus grain N uptake by 50 and 85% at 74000 and 148000 seeds ha^{-1}, respectively (Table 4.17). Within the high N treatment, N cob plus grain uptake was higher at lower seeding rates for hybrid A6499, while no differences were observed among plant densities for hybrid A6517.

At LEX2016, cob plus grain N accumulation was significantly affected by a two-way N rate by seeding rate interaction ($p < 0.0001$) (Table 4.5). The High N, High SR treatment increased cob plus grain N uptake at R3 by 13% compared with the High N, Low SR (Table 4.18).

4.2.4. Grain Nutrient Removal

Grain N uptake at maturity varied significantly by environment (Table 4.6). At LEX2015, High N increased grain N content by 157% compared with Zero N ($p = 0.0423$; Table 4.19).

At LEX2016, significant effects of N rate ($p = 0.0017$) and seeding rate ($p = 0.0258$) were observed on N removal by the grain (Table 4.19). The 390 kg N ha^{-1} rate increased N removed with grain by 196% when contrasted with Zero N. In addition, the greater plant density improved grain N uptake by 11% (Table 4.19).

Grain N uptake at HAR2016 was significantly affected by a two-way N rate by seeding rate interaction ($p = 0.0010$) (Table 4.6). At the Zero N rate, the higher seeding rate did not improve grain N accumulation. Conversely, when the higher N rate was applied, the High SR increased N uptake in the grain at maturity by 20% when compared with Low SR (Table 4.19).

As expected, grain P uptake increased linearly with increasing grain N accumulation for each environment (Figure 4.1A). In the same way, grain K uptake also exhibited a positive linear association with grain N accumulation (Figure 4.1B). All environments exhibited the same tendency.

Evidently, the amount of soil N that is available, together with the uptake and accumulation of this element by the crop, strongly influence P and K accumulation by maize plant communities.

When analyzed on a per plant basis, N grain content was significantly influenced by environment ($p = 0.0028$) (Table 4.7).

Grain N uptake per plant at LEX2015 was significantly affected by N rate ($p = 0.0538$) and seeding rate ($p = 0.0014$). The High N rate increased grain N accumulation per plant by 158% above that observed with the Zero N rate (Table 4.19). On the other hand, High SR negatively impacted grain N per plant, decreasing this parameter by 92% in comparison to the Low SR.

Grain N accumulation per plant at LEX2016 decreased with High SR regardless the N rate, in response to a significant N rate by seeding rate interaction ($p < 0.0001$). High SR decreased grain N accumulation per plant by 86% at the High N rate (Table 4.20).

Nitrogen rate ($p = 0.0021$) and seeding rate ($p < 0.0001$) also significantly influenced grain N removed per plant at HAR2016 (Table 4.7). In this trial, grain N at the High N rate was 58% above that at the Zero N rate. In addition, the increase in seeding rate led to an 88% decline in grain N accumulation per plant (Table 4.20).

As anticipated, grain N accumulation per plant decreased with the High SR, in all environments. However, the application of the High N rate led to an increase in this parameter, as expected.

4.2.5. Whole Plant N Uptake

Total N uptake per hectare was analyzed for both Lexington environments. In this way, environment had a significant effect on whole plant N accumulation ($p = 0.0043$) (Table 4.8).

At both LEX2015 and LEX2016, total N accumulation in aboveground biomass was significantly affected by the two-way interaction of N rate by seeding rate ($p = 0.0481$ and $p < 0.0001$, respectively) and also the N rate by growth stage ($p < 0.0001$ and $p < 0.0001$. respectively) interaction.

Even with these interactions, similar responses to nitrogen uptake occurred, where the highest N accumulations were mostly at R5 and R6 (Tables 4.21 and 4.22). Specifically, whole plant N accumulation at LEX2015 was greatest at R3, R5 and R6 for the Zero N rate and at R5 and R6 when the High N rate was applied. At LEX2016, similar results were obtained with the High N rate, with maximum total N accumulation at R5 and R6 (Table 4.22).

The High N rate increased whole plant N uptake at maturity by 160 and 176% above the Zero N rate when averaged across seeding rates at LEX2015 and LEX2016, respectively.

On the other hand, when averaged across growth stages, whole plant N accumulation at 148,000 plants ha^{-1} was 17% higher when compared to the Low SR at LEX2016. No differences were observed among seeding rates at LEX2015 within the High N rate (Table 4.21, 4.22).

At maturity and within the High N rate, total N accumulation averaged 279 and 247 kg N ha^{-1} at LEX2015 and LEX2016, respectively (Table 4.21, 4.22). Average grain yields were 17 and 18 Mg ha^{-1}, respectively (data shown in Chapter 3).

4.2.6. Nitrogen Deficiency Rating

Nitrogen deficiency in maize plants was observed progressing closer to the ear leaf with High SR and Zero N supply in all environments.

Environment significantly affected N deficiency ratings ($p = 0.0493$). At LEX2015, N rate ($p = 0.0073$) and seeding rate ($p = 0.0090$) treatments significantly influenced this parameter. Nitrogen deficiency was observed to be 198% closer to the ear leaf when N was limiting, compared to the High N rate (Figure 4.2B). High SR, similarly, led to N deficiency being detected 70% closer to the ear leaf when compared to Low SR (Figure 4.2A).

A three-way N rate by seeding rate by hybrid interaction ($p = 0.0036$) significantly affected visible N deficiency at LEX2016 (Table 4.23). At Zero N, the High SR resulted in a 43% higher progression of N deficiency towards the ear leaf than the Low SR. At High SR, the Zero N caused N deficiency to progress 112% higher on the plants compared with High N.

Nitrogen deficiency was also detected higher on the plant in HAR2016 at the High SR and Zero N ($p < 0.0001$). As expected, this treatment combination caused N deficiency to advance significantly higher on maize plants (30%), as opposed to High SR at the High N rate (Figure 4.2C).

Table 4.1. Soil nitrogen concentrations (ppm) for each environment expressed as NH_4-N (ammonium-N), NO3-N (nitrate-N) and total inorganic N.

	Soil N concentrations (ppm)		
Environment	NH_4^+-N	NO_3^--N	Total N
LEX2015			
0-15 cm	3.74	5.90	9.64
15-30 cm	2.74	5.09	7.83
LEX2016			
0-15 cm	0.72	46.88	47.61
15-30 cm	0.51	21.52	22.03
HAR2016			
0-15 cm	0.72	45.39	46.10
15-30 cm	0.47	18.85	19.32

Table 4.2. ANOVA table for stem and leaf N uptake.

Source of variation	stem & leaf N uptake ha^{-1} p-value	Environment	Source of variation	stem & leaf N uptake ha^{-1} p-value
env	<0.0001†	Lexington 2015	n	<0.0001‡
n	<0.0001		hyb	0.9145
env*n	0.8669		n*hyb	0.5313
hyb	0.6966		pop	0.8931
env*hyb	0.4519		n*pop	0.0362
n*hyb	0.2789		hyb*pop	0.2667
env*n*hyb	0.9271		n*hyb*pop	0.7988
pop	0.0946		time	<0.0001
env*pop	0.1673		n*time	<0.0001
n*pop	0.0001		hyb*time	0.9496
env*n*pop	0.6149		n*hyb*time	0.9337
hyb*pop	0.4684		pop*time	0.7328
env*hyb*pop	0.1009		n*pop*time	0.2831
n*hyb*pop	0.4330		hyb*pop*time	0.0329
env*n*hyb*pop	0.2025		n*hyb*pop*time	0.7373
time	<0.0001			
env*time	0.0002	Lexington 2016	n	<0.0001§
n*time	<0.0001		hyb	0.0466
env*n*time	0.1356		n*hyb	0.1413
hyb*time	0.4768		pop	<0.0001
env*hyb*time	0.8013		n*pop	<0.0001
n*hyb*time	0.2608		hyb*pop	0.9336
env*n*hyb*time	0.2863		n*hyb*pop	0.3567
pop*time	0.1917		time	<0.0001
env*pop*time	0.8533		n*time	<0.0001
n*pop*time	0.0130		hyb*time	0.2177
env*n*pop*time	0.8024		n*hyb*time	0.0089
hyb*pop*time	0.0261		pop*time	<0.0001
env*hyb*pop*time	0.0253		n*pop*time	<0.0001
n*hyb*pop*time	0.7659		hyb*pop*time	0.9955
env*n*hyb*pop*time	0.7292		n*hyb*pop*time	0.9991

† ANOVA table for LEX2015 and LEX2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

‡ ANOVA table for LEX2015.

§ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

Table 4.3. ANOVA table for husk N uptake at R5 for LEX2015 and at R3, R5 and R6 at LEX2016.

Environment	Source of variation	R5 husk N uptake ha^{-1} p-value
Lexington 2015	n	0.1207†
	hyb	0.2454
	n*hyb	0.2175
	pop	0.0056
	n*pop	0.1190
	hyb*pop	0.0153
	n*hyb*pop	0.0255

	Source of variation	R3, R5 and R6 husk N uptake ha^{-1} p-value
Lexington 2016	n	<0.0001‡
	hyb	<0.0001
	n*hyb	0.0088
	pop	0.2892
	n*pop	0.2368
	hyb*pop	0.4041
	n*hyb*pop	0.5592
	time	<0.0001
	n*time	<0.0001
	hyb*time	0.0960
	n*hyb*time	0.0860
	pop*time	0.0213
	n*pop*time	0.7615
	hyb*pop*time	0.9792
	n*hyb*pop*time	0.9556

†ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

‡ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

Table 4.4. ANOVA table for cob N uptake at R5 for LEX2015 and at R5 and R6 for LEX2016.

Environment	Source of variation	R5 cob N uptake ha^{-1} p-value
Lexington 2015	n	0.2658 †
	hyb	0.8340
	n*hyb	0.4335
	pop	0.4395
	n*pop	0.8706
	hyb*pop	0.5492
	n*hyb*pop	0.7109

	Source of variation	R5 and R6 cob N uptake ha^{-1} p-value
Lexington 2016	n	<0.0001 ‡
	hyb	0.0150
	n*hyb	0.8458
	pop	<0.0001
	n*pop	<0.0001
	hyb*pop	0.0662
	n*hyb*pop	0.0704
	time	<0.0001
	n*time	0.9053
	hyb*time	0.3024
	n*hyb*time	0.0184
	pop*time	0.0813
	n*pop*time	0.1633
	hyb*pop*time	0.6460
	n*hyb*pop*time	0.7275

†ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

‡ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

Table 4.5. ANOVA table for cob plus grain N uptake at R3.

Source of variation	cob + grain N uptake ha^{-1} p-value	Environment	Source of variation	cob + grain N uptake ha^{-1} p-value
env	0.3882 †	Lexington 2015	n	0.0929 ‡
n	0.0058		hyb	0.2610
env*n	0.0933		n*hyb	0.2070
hyb	0.7259		pop	0.0049
env*hyb	0.2798		n*pop	0.5691
n*hyb	0.6623		hyb*pop	0.1231
env*n*hyb	0.4064		n*hyb*pop	0.0293
pop	0.3143			
env*pop	0.0124	Lexington 2016	n	0.0190 §
n*pop	<0.0001		hyb	0.2906
env*n*pop	0.0355		n*hyb	0.3120
hyb*pop	0.7371		pop	0.7214
env*hyb*pop	0.1977		n*pop	<0.0001
n*hyb*pop	0.7539		hyb*pop	0.1178
env*n*hyb*pop	0.0936		n*hyb*pop	0.6107

†ANOVA table comparing LEX2015 and 2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

‡ ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

§ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

Table 4.6. ANOVA table for grain N uptake at maturity.

Source of variation	grain N uptake ha^{-1} p-value	Environment	Source of variation	grain N uptake ha^{-1} p-value
env	0.0588 †	Lexington 2015	n	0.0423 ‡
n	<0.0001		hyb	0.4906
env*n	0.0685		n*hyb	0.7073
hyb	0.5445		pop	0.9639
env*hyb	0.0548		n*pop	0.1510
n*hyb	0.8925		hyb*pop	0.6156
env*n*hyb	0.2272		n*hyb*pop	0.7550
pop	0.0036			
env*pop	0.1352	Lexington 2016	n	0.0017 §
n*pop	0.0285		hyb	0.0622
env*n*pop	0.7690		n*hyb	0.6611
hyb*pop	0.2717		pop	0.0258
env*hyb*pop	0.8988		n*pop	0.2031
n*hyb*pop	0.8696		hyb*pop	0.3330
env*n*hyb*pop	0.9462		n*hyb*pop	0.9939
		Hardin 2016	n	0.0024 ¶
			hyb	0.1072
			n*hyb	0.6523
			pop	0.0083
			n*pop	0.0010
			hyb*pop	0.5492
			n*hyb*pop	0.1165

†*ANOVA table for all environments. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.*

‡ *ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.*

§ *ANOVA table for LEX2016. All 6 hybrids are included in the analysis.*

¶ *ANOVA table for HAR2016. All 6 hybrids are included in the analysis.*

Table 4.7. ANOVA table for grain N uptake per plant at maturity.

Source of variation	grain N uptake plant^{-1} p-value	Environment	Source of variation	grain N uptake plant^{-1} p-value
env	0.0028 †	Lexington 2015	n	0.0538 ‡
n	<0.0001		hyb	0.3992
env*n	0.1156		n*hyb	0.8632
hyb	0.1606		pop	0.0014
env*hyb	0.0117		n*pop	0.1117
n*hyb	0.6405		hyb*pop	0.9922
env*n*hyb	0.4938		n*hyb*pop	0.7050
pop	<0.0001			
env*pop	0.8192	Lexington 2016	n	0.0010 §
n*pop	0.0825		hyb	0.0436
env*n*pop	0.2156		n*hyb	0.3609
hyb*pop	0.7349		pop	<0.0001
env*hyb*pop	0.0286		n*pop	<0.0001
n*hyb*pop	0.6916		hyb*pop	0.4934
env*n*hyb*pop	0.5859		n*hyb*pop	0.4054
		Hardin 2016	n	0.0021 ¶
			hyb	0.0016
			n*hyb	0.3081
			pop	<0.0001
			n*pop	0.2792
			hyb*pop	0.0142
			n*hyb*pop	0.1363

†ANOVA table for all environments. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.

‡ ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

§ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

¶ ANOVA table for HAR2016. All 6 hybrids are included in the analysis.

Table 4.8. ANOVA table for whole plant N uptake.

Source of variation	whole plant N uptake ha^{-1} p-value	Environment	Source of variation	whole plant N uptake ha^{-1} p-value
env	0.0043†	Lexington 2015	n	0.0002‡
n	<0.0001		hyb	0.6197
env*n	0.2502		n*hyb	0.8641
hyb	0.5102		pop	0.6560
env*hyb	0.1329		n*pop	0.0481
n*hyb	0.8892		hyb*pop	0.3067
env*n*hyb	0.8839		n*hyb*pop	0.4732
pop	0.3099		time	<0.0001
env*pop	0.1037		n*time	<0.0001
n*pop	0.0052		hyb*time	0.9934
env*n*pop	0.8470		n*hyb*time	0.9590
hyb*pop	0.4818		pop*time	0.4516
env*hyb*pop	0.4240		n*pop*time	0.5823
n*hyb*pop	0.8646		hyb*pop*time	0.1368
env*n*hyb*pop	0.2309		n*hyb*pop*time	0.4949
time	<0.0001			
env*time	0.2032	Lexington 2016	n	<0.0001§
n*time	<0.0001		hyb	0.0394
env*n*time	0.0934		n*hyb	0.4667
hyb*time	0.2556		pop	0.0004
env*hyb*time	0.2439		n*pop	<0.0001
n*hyb*time	0.4935		hyb*pop	0.9561
env*n*hyb*time	0.2860		n*hyb*pop	0.6976
pop*time	0.3809		time	<0.0001
env*pop*time	0.8436		n*time	<0.0001
n*pop*time	0.6955		hyb*time	0.0030
env*n*pop*time	0.8413		n*hyb*time	0.0665
hyb*pop*time	0.4729		pop*time	0.1403
env*hyb*pop*time	0.4211		n*pop*time	0.1380
n*hyb*pop*time	0.9180		hyb*pop*time	0.9945
env*n*hyb*pop*time	0.5923		n*hyb*pop*time	0.9928

†ANOVA table for LEX2015 and 2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

‡ ANOVA table for LEX2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

§ ANOVA table for LEX2016. All 6 hybrids are included in the analysis.

Table 4.9. ANOVA table for nitrogen harvest index (NHI).

Source of variation	NHI p-value	Environment	Source of variation	NHI p-value
env	0.0168 ł	Lexington 2015	n	0.8179‡
n	0.0403		hyb	0.6322
env*n	0.0401		n*hyb	0.5829
hyb	0.6838		pop	0.9695
env*hyb	0.4544		n*pop	0.6774
n*hyb	0.7796		hyb*pop	0.4823
env*n*hyb	0.3160		n*hyb*pop	0.6774
pop	0.1687			
env*pop	0.2834	Lexington 2016	n	0.0316§
n*pop	0.7534		hyb	0.2517
env*n*pop	0.5993		n*hyb	0.0034
hyb*pop	0.3538		pop	0.0655
env*hyb*pop	0.5610		n*pop	0.1506
n*hyb*pop	0.2350		hyb*pop	0.5571
env*n*hyb*pop	0.0933		n*hyb*pop	0.2360

ł ANOVA table for all environments. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.

‡ ANOVA table for Lexington 2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

§ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

Table 4.10. ANOVA table for crop growth rate (CGR).

Environment	Source of variation	CGR ($g\ m^{-2}\ day^{-1}$) p-value
Lexington 2015	n	0.0027 †
	hyb	0.5855
	n*hyb	0.6329
	pop	0.8773
	n*pop	0.0180
	hyb*pop	0.6600
	n*hyb*pop	0.1450
Lexington 2016	n	<0.0001‡
	hyb	0.0166
	n*hyb	0.7093
	pop	0.0145
	n*pop	0.0950
	hyb*pop	0.6606
	n*hyb*pop	0.7875

† ANOVa table for Lexington 2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

‡ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis

Table 4.11. ANOVA table for N deficiency rating.

Source of variation	N deficiency rating p-value	Environment	Source of variation	N deficiency rating p-value
env	0.0493 ƚ	Lexington 2015	n	0.0073‡
n	<0.0001		hyb	0.3962
env*n	0.0093		n*hyb	0.2078
hyb	0.6302		pop	0.0090
env*hyb	0.1598		n*pop	0.3663
n*hyb	0.9795		hyb*pop	0.8877
env*n*hyb	0.0714		n*hyb*pop	0.3268
pop	<0.0001			
env*pop	0.1497	Lexington 2016	n	0.0150§
n*pop	0.0828		hyb	0.0548
env*n*pop	0.8515		n*hyb	0.1082
hyb*pop	0.3710		pop	<0.0001
env*hyb*pop	0.9214		n*pop	0.0047
n*hyb*pop	0.7058		hyb*pop	0.5875
env*n*hyb*pop	0.1874		n*hyb*pop	0.0036
		Hardin 2016	n	0.0047¶
			hyb	<0.0001
			n*hyb	0.0002
			pop	<0.0001
			n*pop	<0.0001
			hyb*pop	0.0465
			n*hyb*pop	0.6805

ƚ ANOVA table for all environments. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.

‡ ANOVA table for Lexington 2015. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis.

§ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

¶ ANOVA table for Hardin 2016. All 6 hybrids are included in the analysis.

Table 4.12. ANOVA table for nitrogen internal efficiency (NIE) for LEX2015 and LEX2016.

Source of variation	NIE (kg grain kg⁻¹ N uptake) p-value	Environment	Source of variation	NIE (kg grain kg⁻¹ N uptake) p-value
env	0.6549 †	Lexington 2016	hyb	0.0360‡
hyb	0.1047		pop	0.0016
env*hyb	0.4548		hyb*pop	0.5000
pop	0.0364			
env*pop	0.0449			
hyb*pop	0.9938			
env*hyb*pop	0.5951			

† ANOVA table for LEX2015 and LEX2016. AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments share these hybrids.

‡ ANOVA table for Lexington 2016. All 6 hybrids are included in the analysis.

Table 4.13. Seeding rate and growth stage effects on stem and leaf N uptake per hectare, within N rate, for LEX2015.

LEX2015		seeding rate (seeds ha^{-1})				Mean		p-value‡	
		74000		148000		by growth stage			
N rate (kg N ha^{-1})	Growth Stage	N uptake stem + leaf (kg N ha^{-1})							
0	V7	36.5	B†	37.5	B	37.0		ns*	b§
	V14	88.2	A	53.7	A	70.9		0.0046	b
	R3	75.9	A	65.7	A	70.8		ns	b
	R5	38.3	B	25.1	B	31.7		ns	b
	R6	32.1	B	25.5	B	28.8		ns	b
390	V7	49.6		63.3		56.4	C†	ns	a
	V14	153.0		186.3		169.7	A	ns	a
	R3	159.4		188.3		173.9	A	ns	a
	R5	94.4		89.6		92.0	B	ns	a
	R6	76.7		77.0		76.9	BC	ns	a

†For the low N rate, the growth stage x seeding rate interaction was significant, therefore, means are compared within each seeding rate. Means in the same column with different letters are significantly different ($p \leq 0.10$). Within the high N rate, seeding rate did not interact with growth stage and means comparisons are for each growth stage averaged across seed rate. Means in the same column with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same row within N rate and growth stage with a p value ≤ 0.10 are significantly different.

**ns = not significant at an $\alpha = 0.10$ significance level.*

§ Mean comparison for N rate effect at each growth stage. Means at each growth stage with different letters are significantly different ($p < 0.0001$).

Table 4.14. Seeding rate and growth stage effects on stem and leaf N uptake per hectare, within N rate, for LEX2016.

LEX2016		seeding rate (seeds ha^{-1})				Mean		
		74000		148000		by growth stage		p-value‡
N rate (kg N ha^{-1})	Growth Stage	N uptake stem + leaf (kg N ha^{-1})						
0	V7	13.6		14.7		14.1	D†	ns*
	V14	32.3		30.1		31.2	A	ns
	R3	21.8		19.8		20.8	B	ns
	R5	18.6		13.9		16.2	CD	0.0171
	R6	18.2		18.1		18.1	CB	ns
390	V7	28.7	D†	55.0	D	41.9		<0.0001
	V14	116.8	A	165.6	A	141.2		<0.0001
	R3	110.0	A	139.1	B	124.6		0.0030
	R5	76.7	B	79.7	C	78.2		ns
	R6	46.8	C	37.5	E	42.2		0.0199
		p-value §						
0 vs. 390	V7	0.0252		0.0127				
	V14	0.0008		<0.0001				
	R3	0.0130		<0.0001				
	R5	<0.0001		<0.0001				
	R6	<0.0001		<0.0001				

†For the low N rate, seeding rate did not interact with growth stage (p = 0.6304) and mean comparisons are for each growth stage averaged across seeding rate. Means in the same column with different letters are significantly different (p ≤ 0.10). Within the high N rate, the growth stage x seeding rate interaction was significant (p<.0001), therefore, means are compared within each seeding rate. Means in the same column with different letters are significantly different (p≤0.10).

‡ Means in the same row within N rate and growth stage with a p value ≤ 0.10 are significantly different.

**ns = not significant at a α=0.10 significance level.*

§ Means comparison among N rate treatments within seeding rate for each growth stage with a p-value ≤ 0.10 are significantly different.

Table 4.15. Seeding rate and hybrid effects on R5 husk and cob N uptake per hectare, within N rate.

LEX2015		Hybrid				Mean		Hybrid		Mean
		A6499		A6517		by seeding rate		A6499	A6517	by seeding rate
N rate (kg N ha^{-1})	seeding rate (seeds ha^{-1})	husk N uptake (kg N ha^{-1})						cob n uptake (kg N ha^{-1})		
0	74000	2.8	ns*	2.7	ns	2.8	ns	8.1 ‡	5.9	5.9
	148000	1.6		1.9		1.8		9.1	8.2	8.6
	Mean	2.2		2.3				9.1	7.1	
390	74000	7.4	A †	3.8	A	5.6		13.8	12.2	13.0
	148000	3.1	B	3.8	A	3.4		13.8	17.3	15.5
	Mean	5.2		3.8				13.8	14.7	

†N rate x seeding rate x hybrid interaction for husk N uptake was significant ($p = 0.0255$) for LEX2015. Means in the same column within N rate and hybrid with different letters are significantly different ($p \leq 0.10$).

‡ Cob N uptake at R5 means separation for main effects and interactions were not significant ($p \leq 0.10$).

** ns = not significant ($p \leq 0.10$).*

Table 4.16. Seeding rate effect on husk and cob N accumulations with growth stage.

LEX2016	seeding rate (seeds ha⁻¹)				seeding rate (seeds ha⁻¹)			
	74000		148000		74000		148000	
Growth Stage	husk N uptake (kg N ha⁻¹)				cob N uptake (kg N ha⁻¹)			
R3	9.0	A †	7.8	B	–		–	
R5	2.8	A	2.7	A	3.5	B ‡	4.3	A
R6	2.5	A	3.1	A	4.7	B	6.3	A

†Seeding rate x growth stage interaction for husk N uptake was significant (p = 0.0213) for LEX2016. Means in the same row within growth stage with different letters are significantly different (p ≤ 0.10).

‡ Seeding rate x growth stage interaction for cob N uptake was significant (p = 0.0813). Means in the same row within a growth stage with different letters are significantly different (p ≤ 0.10).

Table 4.17. Seeding rate and hybrid effects on grain plus cob N accumulation per hectare at R3, within N rate.

LEX2015		Hybrid				Mean	
		A6499		A6517		by seeding rate	
N rate (kg N ha⁻¹)	seeding rate (seeds ha⁻¹)	N uptake grain + cob (kg N ha⁻¹)					
	74000	19.9		22.5		21.2	A †
0	148000	14.4		14.0		14.2	B
	Mean	17.1		18.2			
	74000	41.0	A‡	22.6	A	31.8	
390	148000	29.6	B	22.9	A	26.2	
	Mean	35.3		22.7			

†N rate x seeding rate x hybrid interaction for grain + cob N uptake was significant at LEX2015. Means in the same column within N rate and seeding rate with different letters are significantly different (p ≤ 0.10).

‡ Means in the same column within N rate and hybrid with different letters are significantly different (p ≤ 0.10).

Table 4.18. Seeding rate effect on grain plus cob N uptake per hectare at R3 within N rate.

LEX2016	seeding rate (seeds ha^{-1})			
	74000		148000	
N rate (kg N ha^{-1})	grain + cob N uptake (kg N ha^{-1})			
0	18.6	A †	11.8	B
390	48.6	B	54.8	A

†N rate x seeding rate interaction for grain plus cob N uptake was significant at LEX2016. Means in the same row within N rate with different letters are significantly different ($p \leq 0.10$).

Table 4.19. Nitrogen and seeding rate effects on R6 grain N accumulation.

		seeding rate (seeds ha^{-1})					
		74000		148000		Mean	
Environment	N rate (kg N ha^{-1})	grain N uptake (kg N ha^{-1})					
	0	83.9		66.0		75.0	B†
LEX2015	390	183.3		202.3		192.8	A
	Mean	133.6	ns*	134.1			
	0	62.3		68.5		65.4	B†
LEX2016	390	182.9		204.2		193.5	A
	Mean	122.6	B‡	136.3	A		
	0	91.5	A§	88.0	A	89.7	
HAR2016	390	136.6	B	163.4	A	150.0	
	Mean	114.0		125.7			

†Means for the N rate effect on grain N uptake are averaged across seeding rates when the N rate by seeding rate interaction was not significant. Means in the same column with different letters are significantly different ($p \leq 0.10$).

‡ Means for the seeding rate effect on grain N uptake are averaged across N rates when the N rate x seeding rate interaction was not significant. Means in the same row with different letters are significantly different ($p \leq 0.10$).

§ N rate x seeding rate interaction for grain N uptake was significant ($p = 0.0010$). Means in the same row within environment and N rate with different letters are significantly different ($p \leq 0.10$).

**ns = not significant at the $\alpha = 0.10$ significance level.*

Table 4.20. Nitrogen and seeding rate effects on R6 grain N uptake per plant.

		seeding rate (seeds ha^{-1})					
		74000		148000		Mean	
Environment	N rate (kg N ha^{-1})	grain N uptake (g N plant^{-1})					
	0	1.180		0.485		0.833	B †
LEX2015	390	2.738		1.555		2.147	A
	Mean	1.959	A †	1.020	B		
	0	1.007	A ‡	0.536	B	0.772	
LEX2016	390	3.028	A	1.624	B	2.326	
	Mean	2.018		1.080			
	0	1.415		0.648		1.032	B§
HAR2016	390	2.065		1.202		1.634	A
	Mean	1.740	A§	0.925	B		

†Means for N rate and seeding rate effects on grain N uptake when the N rate by seeding rate interaction was not significant for LEX2015. Means in the same row and column with different letters are significantly different ($p \leq 0.10$).

‡ The N rate by seeding rate interaction for grain N uptake was significant ($p < 0.0001$) for LEX2016. Means in the same row within N rate with different letters are significantly different ($p \leq 0.10$).

§ Means for N rate and seeding rate effects on grain N uptake when the N rate by seeding rate interaction was not significant for HAR2016. Means in the same row and column with different letters are significantly different ($p \leq 0.10$).

Table 4.21. Seeding rate and growth stage effects on whole plant N uptake per hectare, within N rate, for LEX2015.

LEX2015		seeding rate (seeds ha^{-1})				Mean			
		74000		148000		by growth stage		p-value‡	
N rate (kg N ha^{-1})	Growth Stage	Whole Plant N Uptake (kg N ha^{-1})							
0	V7	36.5		37.5		37.0	D†	ns*	a §
	V14	88.2		53.7		70.9	C	0.0560	b
	R3	97.1		79.9		88.5	B	ns	b
	R5	123.5		87.0		105.2	A	ns	b
	R6	116.0		91.5		103.8	AB	ns	b
	Mean	92.2	A†	69.9	B				
390	V7	49.6		63.3		56.4	D†	0.0298	a
	V14	153.0		186.3		169.7	C	ns	a
	R3	191.2		214.5		202.9	B	ns	a
	R5	252.7		237.5		245.1	A	ns	a
	R6	260.1		279.2		269.7	A	ns	a
	Mean	181.3	ns	196.2					

†For the low N rate, seeding rate did not interact with growth stage and means comparisons are for each growth stage and seeding rate. Means in the same column or row with different letters are significantly different ($p \leq 0.10$). Within the high N rate, seeding rate did not interact with growth stage and means comparisons are for each growth stage, averaged across seeding rate. Means in the same column with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same row within a N rate and growth stage with a p value ≤ 0.10 are significantly different.

**ns = not significant at the $\alpha = 0.10$ significance level.*

§ Mean comparison for the N rate effect at each growth stage. Means at each growth stage with different letters are significantly different ($p < 0.0001$).

Table 4.22. Seeding rate and growth stage effects on whole plant N uptake per hectare, within N rate, for LEX2016.

LEX2016		seeding rate (seeds ha^{-1})				Mean			
		74000		148000		by growth stage		p-value‡	
N rate (kg N ha^{-1})	Growth Stage	Whole Plant N Uptake (kg N ha^{-1})							
	V7	13.6		14.7		14.1	E†	ns*	b§
	V14	32.3		30.1		31.2	D	ns	b
0	R3	44.1		34.6		39.3	C	0.0039	b
	R5	70.7		60.0		65.3	B	ns	b
	R6	85.8		92.8		89.3	A	ns	b
	Mean	49.3	ns	46.4					
	V7	28.8		55.1		41.9	D†	ns	a
	V14	116.9		165.6		141.2	C	ns	a
390	R3	172.9		206.5		189.7	B	0.0029	a
	R5	235.7		246.2		241.0	A	ns	a
	R6	238.7		254.3		246.5	A	ns	a
	Mean	158.6	B†	185.6	A				

†For the low N rate, seeding rate did not interact with growth stage and means comparisons are for each growth stage averaged across seeding rate. Means in the same column with different letters are significantly different ($p \leq 0.10$). Within the high N rate, seeding rate did not interact with growth stage and means comparisons are for each growth stage and seeding rate. Means in the same column or row with different letters are significantly different ($p \leq 0.10$).

‡ Means in the same row within N rate and growth stage with a p value ≤ 0.10 are significantly different.

**ns = not significant at the α = 0.10 significance level.*

§ Mean comparison for N rate effect at each growth stage. Means at each growth stage with different letters are significantly different ($p < 0.0001$).

Table 4.23. Seeding rate and hybrid effects on R5 nitrogen deficiency rating, within N rate, for LEX2016.

LEX2016		Hybrid												Mean		
		A6499		A6517		DKC62-78RIB		DKC67-72RIB		P0339AM		P2089AM		Growth Stage		N rate
N rate (Kg N Ha⁻¹)	SR (seeds ha⁻¹)	N Deficiency Rating														
0	74000	2.47		1.67		2.07		3.13		1.33		1.47		2.02	A †	b ‡
	148000	1.53		1.13		1.47		1.80		1.33		1.20		1.41	B	b
	Mean	2.00	B †	1.40	C	1.77	CB	2.47	A	1.33	C	1.33	C			
390	74000	3.53	A †	4.53	A	4.27	A	4.20	A	4.27	A	4.47	A	4.21	ns	a
	148000	3.02	A	2.87	B	3.27	B	3.40	A	1.98	B	3.40	B	2.99		a
	Mean	3.28	ns*	3.70		3.77		3.80		3.13		3.93				

†For the low N rate, seeding rate did not interact with hybrid and means comparisons are for each seeding rate and hybrid. Means in the same column or row with different letters are significantly different (p ≤ 0.10). Within the high N rate, the seeding rate by hybrid interaction was significant; therefore, means are compared within each hybrid. Means in the same column with different letters are significantly different (p ≤ 0.10).

‡ Mean comparison for N rate effect at each seeding rate. Means at each growth stage with different letters are significantly different (p ≤ 0.10).

**ns = not significant at the α = 0.10 significance level.*

Table 4.24. Nitrogen rate and hybrid effects on nitrogen harvest index for LEX2016.

LEX2016	Hybrid											
	A6499		A6517		DKC62-78RIB		DKC67-72RIB		P0339AM		P2089AM	
N rate (kg N ha⁻¹)	Nitrogen Harvest Index											
0	0.73	B †	0.75	A	0.73	A	0.75	A	0.69	B	0.70	A
390	0.79	A	0.78	A	0.78	A	0.74	A	0.86	A	0.77	A

†LEX2016 Nitrogen harvest index means separated by N rate for each hybrid across seeding rates. The N rate by hybrid interaction was significant and means are compared within each hybrid. Means in the same column with different letters are significantly different (p ≤ 0.10).

Table 4.25. Seeding rate effect on nitrogen harvest index for LEX2016.

LEX2016		
seeding rate (seeds ha^{-1})	Nitrogen Harvest Index	
74000	0.75	B†
148000	0.77	A

† Means separation for seeding rate effect on Nitrogen Harvest Index are averaged across N rate and hybrid. Means in the same column with different letters are significantly different (p ≤ 0.10).

Table 4.26. Nitrogen and seeding rate effects on crop growth rate (CGR) for LEX2015 and LEX2016.

	N rate (kg N ha^{-1})	seeding rate (seeds ha^{-1})	CGR (g m^{-2} day^{-1})	
LEX2015	0	74000	19.0	C†
		148000	15.7	D
	390	74000	27.2	B
		148000	30.2	A
LEX2016	0	74000	12.9	C‡
		148000	13.5	C
	390	74000	23.9	B
		148000	27.0	A

† N rate by seeding rate interaction for crop growth rate was significant (p = 0.0180) for LEX2015. Means in the same column with different letters are significantly different (p ≤ 0.10).

‡ N rate by seeding rate interaction for crop growth rate was significant (p = 0.0950) for LEX2016. Means in the same column with different letters are significantly different (p ≤ 0.10).

Figure 4.1. Relationship between grain (A) P and N uptake, and (B) grain K and N uptake for each environment, averaged across hybrids.

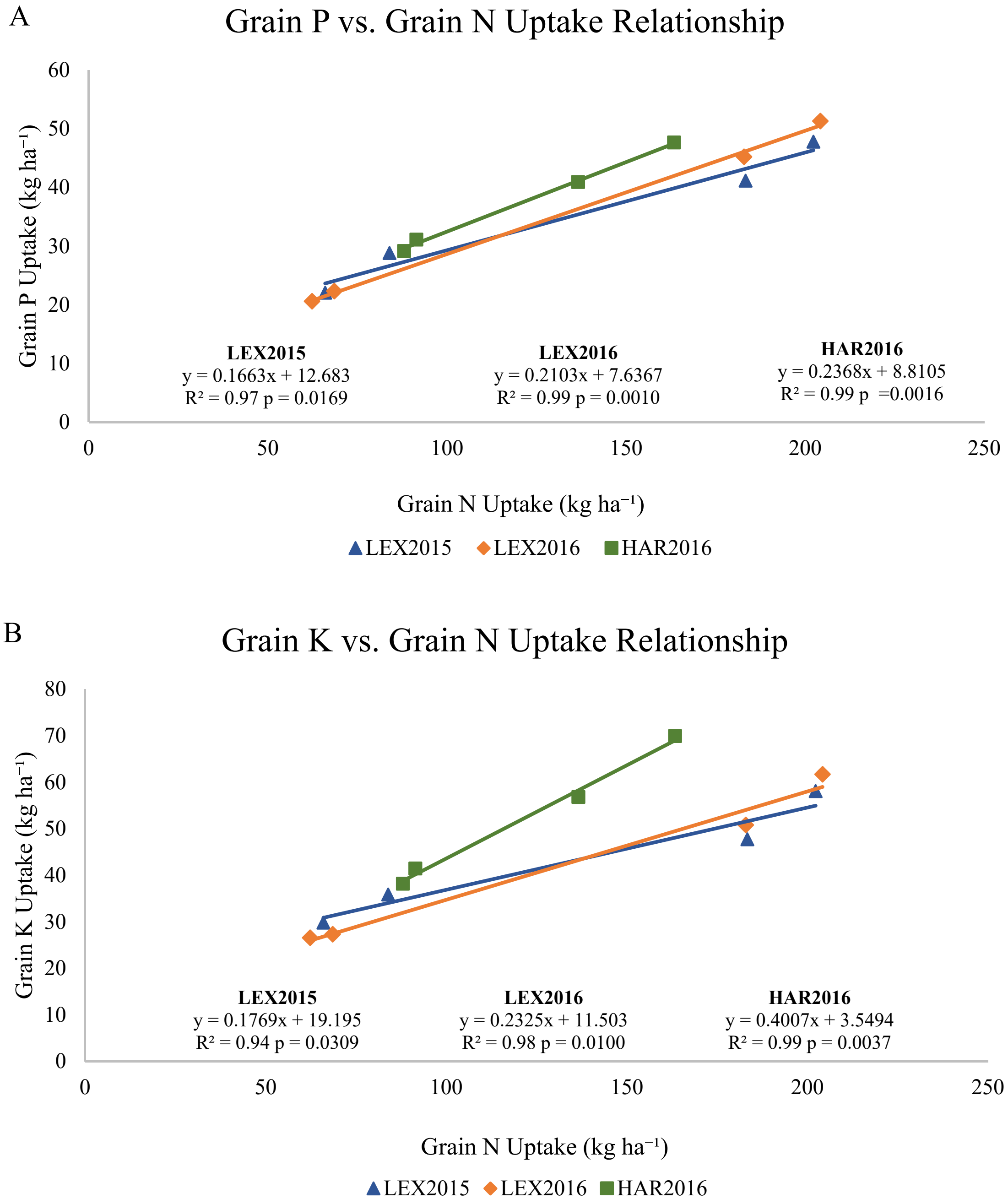


Figure 4.2. Nitrogen deficiency rating evaluated on a scale that designated the ear leaf as "0" and each leaf below the ear leaf with a negative number. Different letters indicate significant differences between treatments (α = 0.1 significance level). (A) and (B) LEX2015, (C) HAR2016.

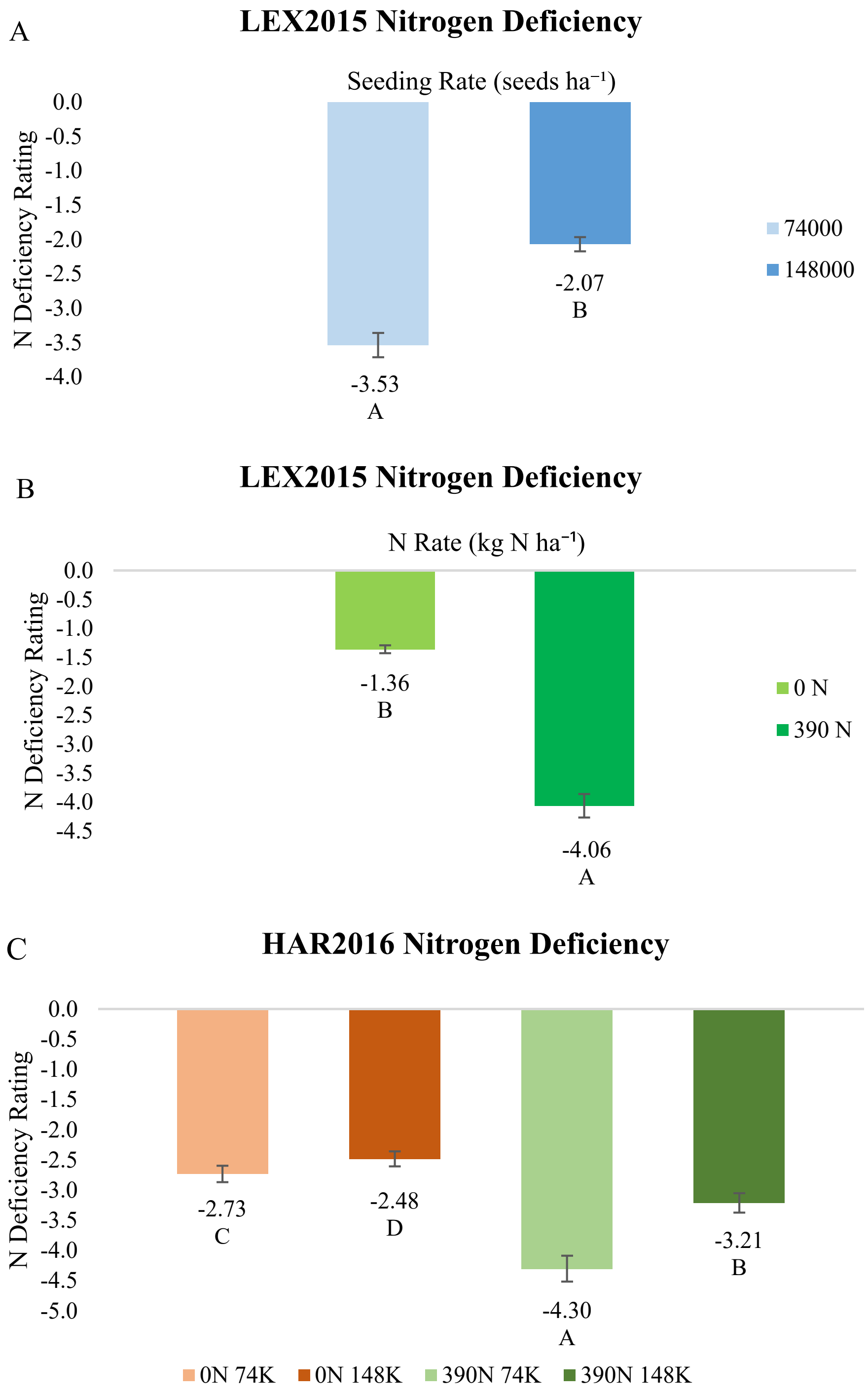
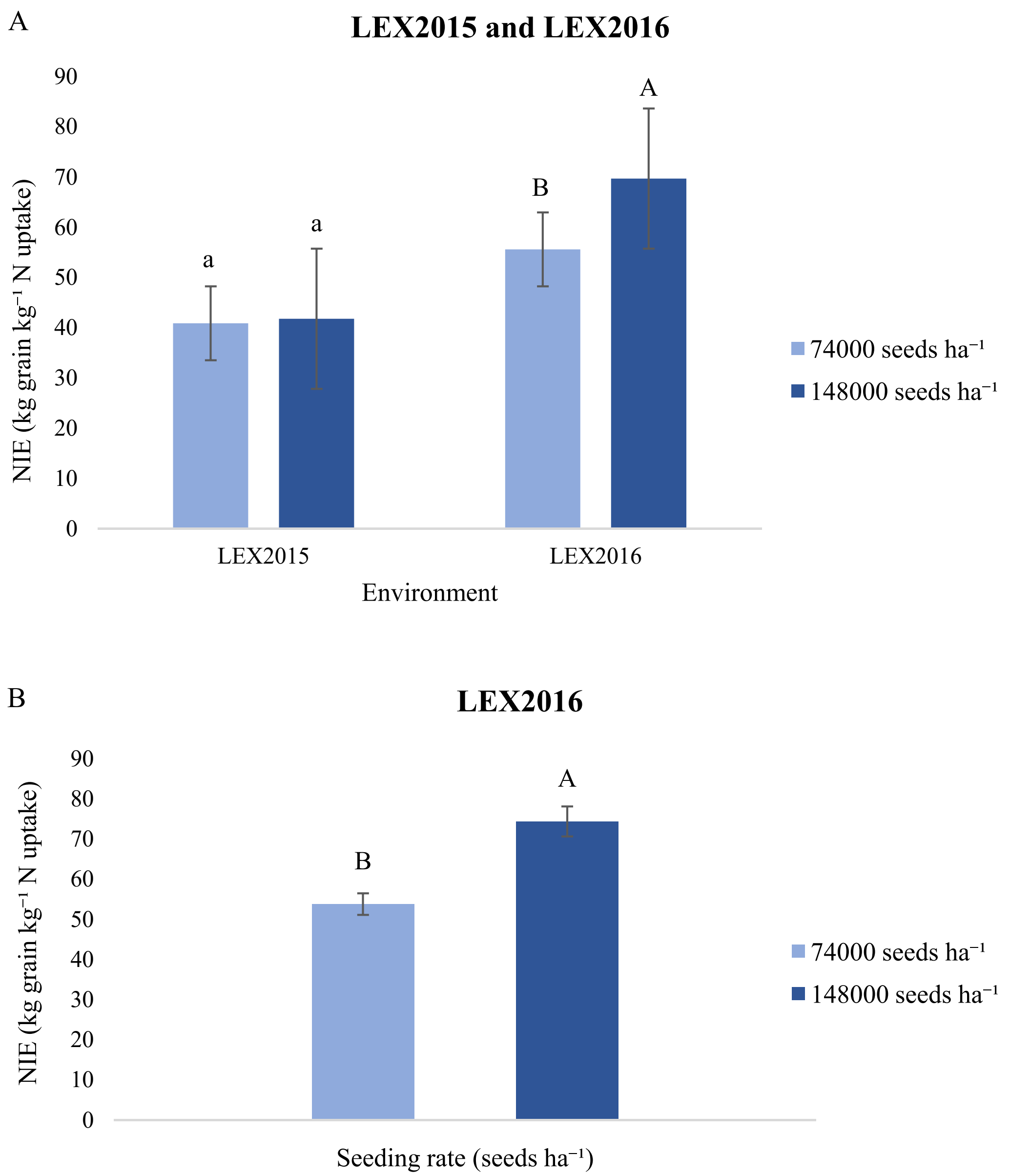


Figure 4.3. Seeding rate effect on nitrogen internal efficiency (NIE) expressed as kg grain yield per kg N uptake. Different letters indicate significant differences between treatments (α = 0.1 significance level). (A) LEX2015 and LEX2106 including 2 hybrids, (B) LEX2016 including all 6 hybrids.



4.3. Discussion

4.3.1. Soil Nitrogen

Overall, before N fertilizer application at V3, the first 15 cm of soil contained the highest amount of available N across all environments and its importance consists mainly in guaranteeing adequate N at planting. LEX2016 and HAR2016 exhibited similar total soil inorganic N values.

Available soil N at LEX2015 was low (<10 ppm total) enough to observe N deficiency symptoms in contrasting treatment combinations across environments.

Phosphorus and potassium soil test results (data not shown) were high at all experiment sites (AGR-1, 2015).

4.3.2. Stem and Leaf N Accumulation Throughout the Growing Season

Stem and leaf nitrogen accumulation was predominantly affected by N rate and seeding rate, with hybrids presenting interactions of magnitude mostly.

As anticipated, the High N rate considerably increased stem and leaf N uptake per hectare, as compared with Zero N, throughout the growing season.

Nitrogen accumulation in the stem and leaf increased gradually, exhibiting maximum values at the V14 and R3 growth stages and then experienced a decline as the season progressed. This temporal pattern was observed under both limiting and non-limiting N rates. However, N accumulation in stem and leaf was always higher with the non-limiting N application. At maturity, fodder N accumulation was 167 and 133% greater at LEX2015 and LEX2016, respectively, with the High N, as compared to the Zero N rate. Other studies had similar observations on N uptake even though their maximum plant densities and N rates were lower (Ciampitti et al., 2013a, Massignam et al., 2009) than this study.

Furthermore, increasing plant density in combination with a non-limiting N supply resulted in greater stem and leaf N accumulation until R3 in LEX2016. At R5, N accumulation was similar for both SRs and at R6, the High SR had 20% lower stem and leaf N accumulation. These observations are consistent with our initial hypothesis. Overall, the High SR accumulated more N per hectare in stem and leaf when supplied with non-limiting N as compared to recommended maize plant densities.

Ciampitti and Vyn, (2011) found similar results, where pre-silking N uptake increased as seeding rates increased. In addition, they found that higher plant populations marginally influenced post-silking N accumulation.

The fact that High SR supplied with non-limiting N rates exhibited greater N accumulation in the stem and leaf component during early vegetative stages and the period bracketing silking (V14-R3) is greatly associated with biomass accumulation dynamics occurring during these developmental stages.

Modern maize hybrids have the potential to increase dry matter production and therefore influence nutrient uptake and assimilation by the crop (Bender et al., 2013a). Furthermore, the increasing trend in past decades of planting higher maize seeding rates has further increased biomass accumulation for this crop (Bender et al. 2012; Boomsma et al. 2009; Below et al. 2007).

Results from this research indicated an increase in biomass production from early vegetative stages that was maximized, on average, at the R3 growth stage. In addition, High SR led to a substantial improvement in stem and leaf dry matter at both N rates (data shown in previous Chapter). Consistent with our results were those found by Bender et al. (2013a) where dry matter production rates were maximized at two identified periods; between growth stages V10 and V14 and also R2 and R3.

According to Ciampitti and Vyn, (2011), the rate at which biomass accumulated was amongst the main factors that influenced N uptake during the vegetative period and after. Nitrogen availability and utilization by plants directly impacts the crop growth rate. Gastal and Lemaire (2002) support the thesis that if sufficient N is available in the soil for plants to use, then N accumulation in plant biomass will be highly related to the crop growth rate. Devienne-Barret et al. (2000) concurred and, in addition, when studying the influence of both crop growth rate and soil nitrogen concentration on N assimilation by crops in the absence of water stress, concluded that N accumulated in fodder can occasionally exceed the minimal N content required for maximum crop growth rates.

With the non-limiting N rate, our results show that the highest crop growth rates were observed when using the highest plant density (Table 4.26). At both LEX2015 and LEX2016 the crop growth rate was 11 and 12.8% higher, respectively, for the High SR as compared to the Low SR. These observations would suggest that when nutrient availability is not a limiting factor, the potential to accumulate higher biomass in these hybrids, when planted at the higher seeding rate is reflected in higher crop growth rates, which leads eventually to increased tissue N uptake.

In agreement with our initial prediction, the High N and High SR increased N accumulation in the stem and leaf fraction when contrasted with N uptake at the Low SR. Apparently, this treatment combination also increased the crop growth rate, thus leading to improved N uptake during early vegetative stages and more importantly, during the critical kernel set period (V14-R3).

Nonetheless, the increase in N accumulation was observed to be diminishing as the crop progressed towards the R3 growth stage. Subsequently, a decrease in stem and leaf content was identified at physiological maturity. The observed decline in stem and leaf N uptake with successive reproductive stages would indicate that N remobilization from vegetative organs to the developing kernels is taking place. Similar observations were reported by Karlen et al., (1988) when providing a non-stressful environment for maize in New Jersey. These authors also described a period of net loss in aboveground N accumulation that extended from VT to R1 and associated this observation to the lack of sink strength at that particular stage of development where the transition from vegetative to reproductive stages occurs.

The higher N accumulation in the fodder fraction, at the High SR and with ample N availability, driven mainly by higher crop growth rates associated with improved biomass accumulation, would therefore be a crucial source of N for redistribution to the developing kernels. Higher grain yields observed with High SR and High N as compared to Low SR and High N would require a subsequent increase in N accumulation by the crop if higher N is expected to be harvested by the grain.

4.3.3. Husk and Cob N Uptake Evolution

Overall, the High SR did not increase N accumulation in the husk, as compared with the Low SR. A declining temporal trend in accumulated husk N was identified as reproductive stages progressed from R3 to maturity. These observations suggest N remobilization from husk tissues to the developing grain.

On the other hand, for the High N rate, N accumulation in the cob fraction at advanced reproductive stages increased 46% with the High SR, as compared to the Low SR, implying that the excess N was accumulating in the cob.

Our observations were consistent with results reported by Sindelar et al. (2013), where cob biomass and N uptake increased with higher N rates of approximately 235 kg ha^{-1}.

Cob N accumulation in this work was largely consistent with the initial hypothesis that cob N accumulation would increase with the High SR and an adequate N supply. Cob N accumulation reflected grain N accumulation, as the High SR, along with the high N rate increased both cob and grain N uptake.

4.3.4. Whole Plant N Accumulation

Whole plant N accumulation at the R6 growth stage averaged 270 and 247 kg N ha^{-1} at LEX2015 and LEX2016, respectively, at the 390 kg N ha^{-1}.

Whole plant N accumulation results observed in this research are comparable to those found in several other studies conducted in recent years. A total N uptake of 286 kg N ha^{-1} at maturity was reported by Bender et al. (2013a) when testing plant populations up to 84,000 plants ha^{-1} in Illinois. Setiyono et al. (2010) reported an average of 232 kg N ha^{-1} accumulated at maturity, with plant densities reaching 110,000 plants ha^{-1}. Likewise, total N uptake at R6 in an irrigated production system was, on average, 262 kg N ha^{-1} according to Djaman et al. (2013).

On the other hand, on average, 63 and 57% of total N accumulation took place by V14 at LEX2015 and LEX2016, respectively, with a non-limiting N supply. These observations would suggest that more than half of the N is accumulated during the vegetative period prior to anthesis. Post-flowering N assimilation by maize, however, is relatively lower and would be partitioned directly to the developing kernels (Bender et al., 2013b).

Grain N accumulation in this study increased at a higher rate, relative to whole plant N, when compared to the findings of Abendroth et al. (2011). In this study, grain N accumulation at R6 with non-limiting N averaged 77 and 81% of whole plant N for Low and High SR, respectively. At the Zero N rate, an average of 72 and 73% of whole plant N was accumulated in the grain at Low and High SR, respectively. Abendroth et al., (2011), reported 67% of total N content was contained in the grain once R6 was reached.

4.3.5. Grain N Removal at Harvest

The amount of grain N removed at harvest varied amongst environments. However, an overall upward trend was observed in grain N accumulation with the High SR and non-limiting N treatment.

Increased biomass production and yield in modern maize hybrids in recent years has resulted from intensified production systems, based on improved management practices. Our results support this tendency as the combined effect of increased SR and a non-limiting N supply increased dry matter accumulation and grain yield, mainly through a greater number of kernels per ha^{-1}. Grain N removal at harvest was likely influenced by both biomass and yield improvements.

As anticipated, a greater amount of N was partitioned to the grain at the High SR and at the High N rate as compared to the Low SR and High N rate treatment combination.

The higher grain N removal found when combining the High SR and High N rate can be related to the greater whole plant N accumulated by V14 (close to silking) in this study. A positive association between crop N uptake at silking and remobilized N from vegetative organs during grain filling has been reported in recent studies (Chen et al., 2015, Ciampitti and Vyn, 2013).

In addition, the described differences in stem and leaf N accumulation between the High and Low SR within High N supply at the R3 growth stage and at later reproductive growth stages, can greatly explain dynamics in N remobilization towards the developing kernels. Similar results were observed by Ciampitti et al. (2013b), who established that N translocation from vegetative tissues taking place later in the growing season would prolong leaf photosynthetic activity and therefore benefit post silking N uptake by the crop.

Based on our results, both remobilization from vegetative organs and prolonged post-silking N uptake post silking served as sources of grain N accumulation. Nitrogen uptake during the reproductive stages following silking was not sufficient to sustain the N demand of the developing ear and thus more N was remobilized from stem and leaf biomass at advanced reproductive stages. Higher plant densities resulting from the higher SR enhanced the translocation process and these plants exhibited lower stem and leaf N accumulation at maturity when compared to those in the Low SR.

Another way of presenting the amount of N translocated from vegetative to reproductive structures is the nitrogen harvest index (NHI; Fageria, 2014). Our results indicate that a significantly greater amount of N was partitioned to the grain with the High SR (NHI = 0.77) as compared to the Low SR (0.75) (Table 4.25). In addition, two out of the six hybrids tested in LEX2016 exhibited a higher NHI at the High N rate as compared to the Zero N rate (Table 4.25).

Alternatively, grain N accumulated per plant exhibited a decline as seeding rate was doubled. However, the High N rate increased the amount of N in the grain but did not dramatically alter N uptake differences between Low and High SR as compared to the Zero N rate. These results can be partially explained by the fact that less kernels per plant were observed as plant densities increased. Nevertheless, the amount of kernels per hectare increased with the higher SR.

According to our results, 202, 204 and 163 kg N ha^{-1} were removed by the grain at harvest in LEX2015, LEX2016 and HAR2016, respectively, when combining High N and High SR. Average grain yields for this treatment combination were 17.9, 19.3 and 13.4 Mg ha^{-1} in each environment, respectively. Bender et al. (2013a), on the other hand, reported 166 kg N ha^{-1} removed by grain with an average yield of 12 Mg ha^{-1} at about 84,000 plants ha^{-1}. Consequently, our results would suggest that an average of 11.3, 10.6 and 12.2 kg N were taken up per Mg grain yield for LEX2015, LEX2016 and HAR2016, respectively, at 148,000 plants ha^{-1}. At 74,000 plants ha^{-1} and with the High N rate, 183, 183 and 137 kg N ha^{-1} were removed by the grain at harvest in LEX2015, LEX2016 and HAR2016, respectively. Average yields for this treatment combination were 17.1, 16.7 and 11.7 Mg ha^{-1} in each environment, respectively.

As a result, an average of 10.7, 10.9 and 11.7 kg N were taken up per Mg grain yield for LEX2015, LEX2016 and HAR2016, respectively. The average kg of N up taken per Mg grain yield was similar for both seeding rates. These results demonstrate the yield potential and stress tolerance to high populations of modern hybrids at the High N and High SR treatment.

On the other hand, when evaluating the Zero N, an overall decline was observed in the amount of N partitioned to the grain at the High SR. Our results show that, on average, 66.0, 68.5 and 88.0 kg N ha^{-1} were removed by the grain at harvest in LEX2015, LEX2016 and HAR2016, respectively, when combining Zero N rate and High SR. Taking under consideration that average grain yields were 9.2, 6.2 and 5.9 Mg ha^{-1} for this combination at each environment, then 7.2, 11.0 and 15.0 kg N were taken up to produce a Mg grain yield for LEX2015, LEX2016 and HAR2016, respectively. Conversely, at the Low SR, 83.9, 62.3 and 91.5 kg N ha^{-1} were harvested by the grain at LEX2015, LEX2016 and HAR2016, respectively. Average grain yields were 9.8, 8.2 and 7.9 Mg ha^{-1} at each environment. As a result, 8.3, 7.7 and 11.6 kg N were taken up per Mg of grain at each environment. These results suggest that grain N uptake was generally lower for the higher SR. Furthermore, an average of 21% reduction in N removed by the grain was observed at 148,000 plants ha^{-1} when N supply was limiting. These observations could be partially explained by the fact that soil mineral N availability at the High SR greatly limited N uptake by the crop (Gastal and Lemaire, 2002) and subsequently N removal by the grain, as compared with the Low SR.

Nevertheless, modern hybrids at the Zero N rate yielded greater than most hybrids tested in the 1950s and 1960s with adequate N, demonstrating greater improvement of current hybrids to take up N in the field (Stevenson and Baldwin, 1969; Nunez and Kamprath, 1969).

At the High SR, our results show higher nitrogen utilization (NIE) as compared with the Low SR for maize. The trend was observed at LEX2016 when analyzing the two hybrids planted at all environments and also when including all six hybrids in the analysis (Figure 4.3A and 4.3B). These observations would suggest that increases in N uptake driven by higher plant densities were enough to improve NIE. Results found by Ciampitti and Vyn, (2011) indicate increased NIE at 79,000 plants ha^{-1} but not at 104,000 plants ha^{-1}, with 330 kg N ha^{-1}. At LEX2015, on the other hand, NIE was similar at both the High and the Low SR (Figure 4.3A). In this environment, grain yield and N uptake at the Zero N rate were greater than at LEX2016, on average. These observations could be largely explained by the greater P and K whole plant uptake observed at LEX2015 at the Zero N rate. Also, these results would suggest that the crop's ability to convert N taken up into grain N with the High N rate was reduced in this environment, thus contributing to the similarities observed in NIE with both SR.

4.3.6. Grain P and K Removal Relationships with Grain N Removal

As anticipated, phosphorus (P) and potassium (K) removed with the grain at maturity responded to N availability. High N increased both P and K grain removal across all environments evaluated (data not shown). Phosphorus grain accumulation exhibited higher values as compared to K accumulation, mainly explained by the greater partitioning of P from vegetative to reproductive organs (Ciampitti et al., 2013c) and the fact that it is accumulated during grain fill. Ziadi et al., (2007) described a linear relationship between whole plant N and P concentrations and attributed the effect of higher N rates on P concentrations to enhanced root growth and nutrient absorption.

In addition, higher whole plant accumulation of P and K has been associated with the greater biomass, total N uptake and yield produced by higher yielding hybrids together with high input systems (Bender et al., 2013b; Ciampitti et al., 2013c).

4.3.7. Nitrogen Deficiency Observed at Reproductive Growth Stages

As a general trend, increased seeding rates lead to the development of N deficiency symptoms in leaves closer to the ear leaf. These observations were consistent across all environments evaluated.

Given nitrogen's high mobility within the plant once assimilated (Bender et al., 2013a), remobilization to grain can be more dynamic if required during the grain filling period.

Nutrient translocation in the developing kernel is expected to start no sooner than R2, once the transition from vegetative to reproductive growth occurs (Abendroth et al., 2011; Bender et al., 2013a). At this point in the growing season, kernel development initiates as does grain nutrient accumulation. Subsequently, grain nutrient accumulation exhibits a linear increase during reproductive growth, during which N is still partitioned to the developing grain, until R5.

Clearly, and as expected, High SR combined with Zero N increased visible N deficiency in maize plants. On average, N deficiency in these cases was observed within one to two leaves below the ear leaf. These observations represent N being translocated from leaf tissues to grain.

As described by Ciampitti et al. (2013b), during reproductive stages N is mostly remobilized from stem and older leaves lower in the plant. Nitrogen supply influences the process of remobilization, and, our results show that the High N rate reduced the progression of observable leaf N deficiency upwards towards the ear leaf. On average, when combining the High SR and the High N rate, three leaves below the ear leaf showed no sign of N deficiency. These results suggest that N is still available for translocation during kernel development at advanced reproductive stages in the crop grown in this treatment.

4.4. Summary

As maize seeding rates have experienced an upward trend in recent years, interest has focused on evaluating and thus updating nutrient assimilation, remobilization and removal patterns within modern maize management systems.

Results from this research indicate greater N accumulation in the stem and leaf fraction when combining the high seeding rate with the high N rate. In addition, higher crop growth / biomass accumulation rates were associated with this treatment.

Nitrogen internal efficiency was increased with the higher seeding rate. Similar results were identified for the amount of nitrogen partitioned to the grain (NHI) as plant densities were doubled.

Ultimately, grain N removal was increased when the high input treatment combination was provided. The expected outcome can be attributed both to the N remobilization process as well as to the increased and sustained N uptake during reproductive growth stages that is characteristic of modern maize hybrids.

Chapter 5

5. Conclusions

High input management systems in conjunction with modern maize hybrids characterized by higher nutrient use efficiency and tolerance to diverse stresses can improve grain yield.

Confirming our initial hypothesis, high maize seeding rates achieved by decreasing row spacing lead to an increase in grain yield when supplied with non-limiting N in Kentucky. Furthermore, the grain yield component that best explained yield improvement was kernel number ha^{-1}. Kernels ha^{-1} was expected to increase at High SR combined with High N.

Conversely, although expected, High SR reduced the number of kernels ear^{-1}. Moreover, our hypothesis was not fully sustained since the High N rate did not improve kernels ear^{-1} at the High SR, as estimated. Kernel number per ear was most influenced by kernels per row. High N increased kernels row^{-1}, still, the effect of High SR reduced this parameter. Therefore, potential kernel number was possibly limited by a factor other than N availability at extreme seeding rates.

Kernel weight was expected to be greater with the High N rate and marginally decrease with High SR. Results relatively confirmed this hypothesis since kernel mass declined with High SR as compared with Low SR. However, the High N rate did not increase kernel weight, restricting further yield increases.

Evidently, the increase in grain yield at High SR and High N was essentially a function of an increment in kernel number ha^{-1}, with a final kernel weight adjustment to balance out such kernel number improvement.

Stem and leaf N content was projected to increase with the joined effect of High SR and high N. In effect, N accumulated in stem and leaf confirmed the initial hypothesis. Increasing plant densities in combination with non-limiting N supply resulted in greater stem and leaf N content per hectare until grain filling.

Nitrogen deficiency, as expected, was observed closer to the ear leaf as High SR and limiting N were combined. Nonetheless, visible N deficiency in maize plants was observed within one to two leaves below the ear leaf.

Cob N content was consistent with the initial hypothesis as it reflected grain N content as seeding rates increased and N rate was high. However, husk N content remobilized quickly from husks regardless of seed rate or N rate.

At maturity, N accumulated in grain was estimated to increase with High SR and High N. Results confirmed that expectation. As anticipated, P and K grain content was increased with higher N accumulation. In addition, grain N content per plant, as expected, was reduced as seeding rates increased. High N did not increase grain N per plant at High SR, primarily as a result of less kernels per plant at higher densities.

The substantial increase in seeding rates resulted in a higher N utilization (NIE), in comparison with low maize seeding rates. Consequently, increases in N uptake driven by higher plant densities were sufficient to improve NIE.

Ultimately, High SR increased the number of kernels harvested per hectare, improved the crop's nitrogen utilization and the amount of N partitioned to the grain per unit area. As a result, maize grain yield was increased as seeding rates were doubled from 74,000 to 148,000 plants ha^{-1} and available N was non-limiting. However, a reduction in kernels per ear and kernel weight as seeding rates increased was still observed under the High N supply, restricting additional yield increases. Subsequently, maize plant densities will preserve the current upward trend if such grain yield components remain inflexible under high input management systems of production. Likely, genetic developments should then focus on kernel mass and kernels per plant for future maize grain yield improvement.

Appendix

Table A.1. ANOVA table summarizing sources of variation when comparing all environments. A α=0.10 significance level was used.

Sources of variation	Grain Yield	Rows Ear^{-1}	Kernels Row^{-1}	Kernels Ear^{-1}	Kernels Ha^{-1}	Kernel Weight	Ear Length †	Tip Back Length †
ANOVA	<.0001	0.0003	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001
Env	0.0022	0.1405	0.3068	0.2299	0.1365	0.2099	0.0448	0.0006
N	<.0001	0.0116	0.0005	0.0005	0.0009	0.0003	<.0001	0.0052
Env*N	0.0002	0.0690	0.5638	0.2417	0.1050	0.0138	0.0272	0.0185
Hyb ‡	0.1307	0.0213	0.2390	0.0984	0.5785	0.0887	<.0001	<.0001
Env*Hyb	0.4416	0.0781	0.0943	0.0504	0.0842	0.4588	0.0212	<.0001
N*Hyb	0.5113	0.1180	0.5397	0.4378	0.7082	0.0997	0.3412	0.0371
Env*N*Hyb	0.1243	0.3941	0.2392	0.3307	0.8387	0.2519	0.3240	0.0409
Pop	0.0099	0.0001	<.0001	<.0001	0.0003	<.0001	<.0001	0.0012
Env*Pop	0.1480	0.1677	0.7830	0.3030	0.5522	0.3140	0.7743	0.3291
N*Pop	<.0001	1.0000	0.0291	0.1665	0.0164	0.0175	0.0068	0.0020
Env*N*Pop	0.0186	0.2783	0.6382	0.3857	0.6721	0.0117	0.0779	0.3556
Hyb*Pop	0.3018	0.5716	0.7646	0.8124	0.3984	0.3381	0.0206	0.0022
Env*Hyb*Pop	0.4510	0.3230	0.9774	0.9010	0.3700	0.9260	0.9327	0.1968
N*Hyb*Pop	0.0104	0.2626	0.8158	0.6499	0.8379	0.2185	0.3103	0.0110
Env*N*Hyb*Pop	0.4887	0.3616	0.4514	0.2619	0.2949	0.0880	0.3968	0.8502

† Lexington 2016 and Hardin 2016 data for this parameter.

‡ AgriGold A6499STXRIB and AgriGold A6517VT3PRIB are included in the analysis since all environments test these hybrids.

Table A.2. ANOVA table summarizing sources of variation when comparing 2016 environments. A α=0.10 significance level was used.

Sources of variation	Grain Yield	Rows Ear^{-1}	Kernels Row^{-1}	Kernels Ear^{-1}	Kernels Ha^{-1}	Kernel Weight	Ear Length Ɨ	Tip Back Length Ɨ
ANOVA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Env	<.0001	0.1554	0.4518	0.0876	0.1050	0.6130	0.0448	0.0006
N	<.0001	0.0008	<.0001	<.0001	<.0001	0.0008	<.0001	0.0052
Env*N	<.0001	0.0434	0.0260	0.0176	0.0008	0.0039	0.0272	0.0185
Hyb Ɨ	<.0001	<.0001	0.0002	<.0001	0.0214	0.0004	<.0001	<.0001
Env*Hyb	0.2924	0.2184	0.0055	0.0123	0.0554	0.5491	0.0212	<.0001
N*Hyb	0.0529	0.0451	0.1950	0.0817	0.1613	0.1818	0.3412	0.0371
Env*N*Hyb	0.5992	0.8333	0.2387	0.5425	0.9441	0.0567	0.3240	0.0409
Pop	0.7074	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012
Env*Pop	0.1252	0.0270	0.8406	0.1754	0.0515	0.8817	0.7743	0.3291
N*Pop	<.0001	<.0001	0.0005	0.0021	<.0001	<.0001	0.0068	0.0020
Env*N*Pop	0.0928	0.5334	0.2591	0.4043	0.9656	0.0001	0.0779	0.3556
Hyb*Pop	<.0001	<.0001	0.1074	0.0046	0.3258	0.0327	0.0206	0.0022
Env*Hyb*Pop	0.4053	0.9548	0.7453	0.8367	0.3652	0.0023	0.9327	0.1968
N*Hyb*Pop	0.0079	0.0018	0.2825	0.1873	0.0609	0.0150	0.3103	0.0110
Env*N*Hyb*Pop	0.8450	0.1392	0.5930	0.3481	0.5712	0.4265	0.3968	0.8502

Ɨ All 6 hybrids are included in the analysis.

Table A.3. N Rate effect on Grain Yield, Rows Ear^{-1}, Kernels Row^{-1}, Kernel Ear^{-1}, 10^6 Kernels Ha^{-1} and 1000 Kernel Weight within Environment, Hybrid and Seeding Rates.

ENV.	Hybrid	SR (K seeds ha^{-1})	N Rate (kg/ha)		p-value†	N Rate (kg/ha)		p-value†	N Rate (kg/ha)		p-value†	N Rate (kg/ha)		p-value†	N Rate (kg/ha)		p-value†	N Rate (kg/ha)		p-value†
			0	390		0	390		0	390		0	390		0	390		0	390	
			Yield (Mg Ha^{-1})			Rows Ear^{-1}			Kernels Row^{-1}			Kernel Ear^{-1}			10^6 Kernels Ha^{-1}			1000 Kernel Wt. (g)		
Lexington 2015	A6499	74	10.3‡	16.9	0.0016	14.5‡	16.5	<.0001	36.5‡	43.0	0.1444	532‡	714	0.0707	37.0‡	49.0	0.3743	247‡	326	0.3574
		148	8.9	18.5	0.0005	14.5	16.0	0.2048	25.5	37.0	0.1881	365	595	0.1706	47.0	66.5	0.2048	265	304	0.3903
	A6517	74	9.0	17.3	0.0672	16.0	15.5	0.7952	38.5	40.5	<.0001	609	634	0.7613	42.0	41.0	0.7048	256	338	0.1476
		148	11.1	17.2	0.0079	15.0	15.0	1.0000	26.0	36.5	0.1488	386	564	0.0214	42.5	77.5	0.0903	208	344	0.2103
		Mean	9.8	17.5	0.0004	15.0	15.8	0.1306	31.6	39.3	0.0114	473	627	0.0012	42.1	58.5	0.0146	244	328	0.0012
Lexington 2016	A6499	74	8.3	15.2	0.0062	15.3	18.3	0.1885	31.0	43.6	0.0247	474	796	0.0831	41.0	64.0	0.1099	263	343	0.0339
		148	6.9	19.1	0.0034	15.0	16.0	0.2254	20.3	34.3	0.0938	304	551	0.0948	45.0	84.3	0.0612	263	279	0.2809
	A6517	74	7.6	17.7	0.0003	17.6	19.0	0.1835	40.0	44.6	0.6084	716	855	0.4182	59.0	79.0	0.2866	270	325	0.0324
		148	7.0	20.6	0.0002	16.0	17.6	0.1296	29.0	37.6	0.3273	477	667	0.2957	63.3	88.3	0.4100	246	270	0.3238
	DKC62	74	8.1	15.7	0.0006	14.6	15.6	0.2254	30.6	41.3	0.0068	456	640	0.0141	39.3	69.3	0.0328	270	381	0.0033
		148	6.8	18.7	0.0001	12.3	15.3	0.0955	22.0	36.3	0.0070	268	560	0.0047	36.0	80.7	0.0195	264	284	0.4582
	DKC67	74	9.7	17.5	0.0001	16.3	17.0	0.1835	35.3	42.0	0.0814	579	721	0.0844	50.0	66.3	0.0768	255	348	0.0492
		148	6.6	19.8	0.0017	12.6	16.6	0.1201	25.6	38.6	0.1333	332	640	0.1138	39.6	99.3	0.0483	270	279	0.5886
	P0339	74	7.0	14.5	0.0009	16.6	17.3	0.4226	30.0	45.6	0.0005	494	796	0.0147	42.3	73.0	0.0341	256	322	0.005
		148	4.0	18.6	0.0008	10.6	15.3	0.0051	21.6	36.3	0.0066	233	564	0.0040	30.3	77.3	0.0360	247	258	0.0533
	P2089	74	8.8	19.5	0.0071	15.6	17.6	0.0742	33.0	50.3	0.0070	517	895	0.0069	41.6	77.0	0.0851	233	381	0.0218
		148	6.0	19.1	0.0100	12.3	16.3	0.0202	20.0	36.6	0.0554	250	603	0.0304	35.3	86.3	0.0693	253	278	0.2091
		Mean	7.2	18.0	0.0001	14.6	16.8	<.0001	28.2	40.6	<.0001	425	691	<.0001	43.6	78.7	<.0001	258	312	<.0001
Hardin 2016	A6499	74	8.5	11.7	0.0375	17.0	17.0	1.0000	37.0	39.5	0.3615	621	669	0.4244	58.0	60.2	0.7190	291	309	0.0377
		148	6.0	13.8	0.0178	15.5	16.2	0.4444	24.0	32.5	0.0057	377	524	0.0166	53.5	71.7	0.0386	276	265	0.5792
	A6517	74	7.9	12.1	0.0019	17.0	16.7	0.7177	33.2	41.5	0.0249	559	698	0.0328	43.5	50.7	0.1666	265	298	0.0421
		148	7.5	14.7	0.0049	16.5	16.7	0.6376	23.2	32.0	0.0027	381	533	0.0108	55.7	68.5	0.0331	228	267	0.1381
	DKC62	74	7.8	10.2	0.0147	15.0	15.2	0.7177	32.7	38.5	0.0743	487	600	0.1288	37.5	54.0	0.0071	296	317	0.1025
		148	7.3	13.2	0.0070	13.5	15.2	0.0354	24.5	31.7	0.0623	330	478	0.0485	48.2	67.2	0.0845	290	291	0.9579
	DKC67	74	7.3	12.5	0.0006	15.0	15.5	0.6638	37.2	39.2	0.3429	549	612	0.0468	45.0	44.7	0.9284	301	353	0.1159
		148	5.2	13.0	0.0035	13.7	14.7	0.0917	23.7	35.7	0.0004	326	527	0.0027	47.5	74.2	0.0282	269	250	0.3044
	P0339	74	7.2	10.0	0.1900	16.2	17.2	0.4226	32.0	41.3	0.0604	519	695	0.0102	42.2	49.2	0.2057	286	280	0.5417
		148	3.5	12.6	0.0293	10.7	15.7	0.0377	21.2	34.2	0.0390	232	518	0.0347	30.0	78.0	0.0179	284	268	0.3613
	P2089	74	8.7	13.4	0.0131	15.0	16.7	0.2126	41.7	47.0	0.1018	615	785	0.0315	47.2	57.7	0.1788	304	322	0.4667
		148	5.8	13.0	0.0122	13.5	14.7	0.0154	28.5	39.7	0.0038	388	597	0.0001	52.0	84.0	0.0101	251	239	0.4610
		Mean	6.9	12.5	<.0001	14.9	16.0	0.0444	29.9	37.7	<.0001	449	603	<.0001	46.7	63.3	0.0025	278	288	0.1609

† Significant values ($p \leq 0.10$) are highlighted.

‡ Means in the same row with a p-value ≤ 0.10 are significantly different.

Table A.4. Seed Rate effect on Grain Yield, Rows Ear^{-1}, Kernels Row^{-1}, Kernel Ear^{-1}, 10^6 Kernels Ha^{-1} and 1000 Kernel Weight within Environment, Hybrid and N Rate.

ENV.	Hybrid	N Rate (kg/ha)	SR (K seeds/ha)		p-value†	SR (K seeds/ha)		p-value†	SR (K seeds/ha)		p-value†	SR (K seeds/ha)		p-value†	SR (K seeds/ha)		p-value†	SR (K seeds/ha)		p-value†
			74	148		74	148		74	148		74	148		74	148		74	148	
			Yield (Mg Ha^{-1})			Rows Ear^{-1}			Kernels Row^{-1}			Kernel Ear^{-1}			10^6 Kernels Ha^{-1}			1000 Kernel Wt. (g)		
Lexington 2015	A6499	0	10.3‡	8.9	0.0290	14.5‡	14.5	1.0000	36.5‡	25.5	0.1695	532‡	365	0.2378	37.0‡	47.0	0.3440	247‡	264	0.8138
		390	16.9	18.5	0.0781	16.5	16.0	0.5000	43.0	37.0	0.4423	714	595	0.4677	49.0	66.5	0.1602	326	304	0.6443
	A6517	0	9.4	10.4	0.4372	16.0	15.0	0.5000	38.5	26.0	0.1738	609	386	0.2617	42.0	42.5	0.7952	255	208	0.1349
		390	17.3	17.2	0.9566	15.5	15.0	0.5000	40.5	36.5	0.1560	634	564	0.2489	41.0	77.5	0.0261	338	344	0.9263
		Mean	13.4	13.7	0.6056	15.6	15.1	0.2746	39.6	31.3	0.0082	622	478	0.0012	42.3	58.4	0.0148	292	280	0.5659
Lexington 2016	A6499	0	8.2	6.7	0.2166	15.3	15.0	0.7418	31.0	20.3	0.0395	474	304	0.1389	41.0	45.0	0.5432	263	263	1.0000
		390	15.2	19.1	0.0157	18.3	16.0	0.1181	43.6	34.3	0.1729	796	551	0.0247	64.0	84.3	0.2051	343	279	0.0073
	A6517	0	7.6	7.0	0.0809	17.6	16.0	0.1296	40.0	29.0	0.1349	716	477	0.0892	59.0	63.3	0.8391	270	246	0.0809
		390	17.7	20.6	0.0045	19.0	17.6	0.1835	44.6	37.6	0.0067	855	667	0.0206	79.0	88.3	0.5081	325	270	0.0488
	DKC62	0	8.1	6.8	0.0113	14.6	12.3	0.1181	30.6	22.0	0.0429	456	268	0.0174	39.3	36.0	0.6878	270	264	0.6401
		390	15.7	18.7	0.0104	15.6	15.3	0.6667	41.3	36.3	0.0820	640	560	0.0941	69.3	80.6	0.0399	381	284	0.0247
	DKC67	0	9.7	6.6	0.0205	16.3	12.6	0.0533	35.3	25.6	0.0713	579	332	0.0324	50.0	39.6	0.0580	255	270	0.5304
		390	17.5	19.8	0.0774	17.0	16.6	0.6667	42.0	38.6	0.0377	721	640	0.1193	66.3	99.3	0.0512	348	279	0.0644
	P0339	0	7.0	4.0	0.0320	16.6	10.6	<.0001	30.0	21.6	0.0110	494	233	0.0008	42.3	30.3	0.1873	256	247	0.5905
		390	14.5	18.6	0.0233	17.3	15.3	0.1835	45.6	36.3	0.0013	796	564	0.0373	73.0	77.3	0.7115	322	258	0.0759
	P2089	0	8.8	6.0	0.0313	15.6	12.3	0.1296	33.0	20.0	0.1215	517	250	0.0868	41.6	35.3	0.5640	233	253	0.3306
		390	19.3	19.1	0.8481	17.6	16.3	0.0572	50.3	36.6	0.0213	895	603	0.0059	77.0	86.3	0.3899	381	278	0.0360
		Mean	12.5	12.7	0.1785	16.7	14.7	<.0001	38.9	29.9	<.0001	662	454	<.0001	58.5	63.8	0.0615	304	266	<.0001
Hardin 2016	A6499	0	8.5	6.0	0.0130	17.0	15.5	0.0138	37.0	24.0	0.0221	621	377	0.0190	58.0	53.5	0.5045	291	276	0.3318
		390	11.7	13.8	0.0426	17.0	16.2	0.3189	39.5	32.5	0.0123	669	524	0.0130	60.2	71.7	0.2077	309	265	0.0346
	A6517	0	7.9	7.5	0.6763	17.0	16.5	0.1817	33.2	23.5	0.0334	559	381	0.0256	43.5	55.7	0.1038	265	228	0.0087
		390	12.1	14.7	0.0162	16.7	16.7	1.0000	41.5	32.0	0.0016	698	533	0.0014	50.7	68.5	0.0350	298	267	0.0197
	DKC62	0	7.8	7.3	0.4869	15.0	13.5	0.0138	32.7	24.5	0.0592	487	330	0.0486	37.5	48.2	0.1235	296	290	0.6182
		390	10.2	13.2	0.0008	15.2	15.2	1.0000	38.5	31.7	0.0124	600	478	0.0293	54.0	67.2	0.0207	317	291	0.0304
	DKC67	0	7.3	5.2	0.1280	15.0	13.7	0.0796	37.2	23.7	0.0002	549	326	0.0009	45.0	47.5	0.5240	301	269	0.0668
		390	12.5	13.0	0.6947	15.5	14.7	0.5195	39.2	35.7	0.1018	612	527	0.0737	44.7	74.2	0.0126	353	250	0.0253
	P0339	0	7.2	3.5	0.0133	16.2	10.7	0.0079	32.0	21.2	0.0159	519	232	0.0008	42.2	30.0	0.0641	286	284	0.8103
		390	10.3	12.5	0.0629	16.6	15.6	0.4226	41.6	33.3	0.0202	684	506	0.0833	50.6	75.6	0.0549	282	271	0.3949
	P2089	0	8.7	5.8	0.0304	15.0	13.5	0.2967	41.7	28.5	0.0130	615	388	0.0322	47.2	52.0	0.6676	304	251	0.0595
		390	13.4	13.0	0.5827	16.7	14.7	0.0163	47.0	39.7	0.0485	785	597	0.0295	57.7	84.0	0.0307	322	239	0.0280
		Mean	9.8	9.6	0.2233	16.1	14.7	<.0001	38.4	29.2	<.0001	616	433	<.0001	49.3	60.7	<.0001	302	265	<.0001

† Significant values ($p \leq 0.10$) are highlighted.

‡ Means in the same row with a p-value ≤ 0.10 are significantly different.

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Vita

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